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The effect of water temperature and salinity on recovery from exercise induced muscle damage

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AUTHORS DECLARATION

"I Amy Campbell, declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

The effect of water temperature and salinity on recovery from exercise induced muscle damage

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THE EFFECT OF WATER TEMPERATURE AND SALINITY ON RECOVERY FROM EXERCISE INDUCED MUSCLE DAMAGE

ABSTRACT

Water immersion strategies are commonly employed to reduce symptoms of exercise induced muscle damage (EIMD). However, little research has established whether recovery is stimulated via effects of hydrostatic pressure or water temperature (Leeder et al., 2012).This study investigated the effects of four immersion protocols of differing temperature and hydrostatic pressures (control (CON), cold water immersion (CWI), thermo-neutral water immersion (TWI) and thermo-neutral saline immersion (TSI) on recovery from EIMD. Twenty-five recreationally active males participated in the study. Participants completed 5 X 8 min of downhill running (-10%, 60% max treadmill velocity) separated by 2 min rest to induce EIMD. Within 30 min post exercise participants were randomly assigned to either: CWI (15 min, 10-15°C), TWI (15 min, ~35°C), TSI (15 min, ~35°C, 30% salinity) or a CON (15 min seated rest). Anthropometric measures, circumference of left (LL) and right leg (RL), cross sectional area (CSA) of rectus femoris via ultra sound scans of LL and RL, countermovement jumps (CMJ), assessment of maximal voluntary contractions (MVC), pain scales, recovery scales, creatine kinase (CK) and blood lactate were obtained across 6 time points (familiarisation up to 72 h post EIMD). Muscle damaging exercise resulted in a significant reduction of CMJ in CON from pre-trials to 24 h post (-10.0 ± 6.7%, $p = 0.001$), and a significant increase for RL (1.1 ± 0.7%, $p = 0.02$) and LL (1.1 ± 0.6%, $p = 0.03$) circumference from pre trials to 24 h post. CK increased significantly for CON and TSI groups between pre-trials and 24 h post (121.3 ± 28.5%, $p = 0.001$; 130.3 ± 95.0%, $p < 0.001$) respectively, however TSI group demonstrated a significant reduction between 24 h and 48 h post ($p = 0.001$). No significant interaction effect was present between groups across measures of LL and RL circumference, CK, MVIC, CMJ and pain scales ($P > 0.05$). Although the early indications from percentage change in performance may reflect a greater effect of a CWI protocol over TSI, the lack of statistical significance across variables provides little indication to whether recovery is stimulated primarily via temperature of the water or hydrostatic pressure. Further, investigations into the effect of a TSI protocol on recovery on a larger sample size, and the effects of TSI on training adaptation should be considered to evaluate the effectiveness of TSI as a recovery strategy.

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LIST OF ABBREVIATIONS

The following abbreviations are used throughout this thesis.

EIMD	Exercise induced muscle damage
CWI	Cold water immersion
TWI	Thermo-neutral water immersion
TSI	Thermo-neutral saline immersion
CMJ	Countermovement jump
CK	Creatine kinase
BLa	Blood Lactate
MVIC	Maximal voluntary isometric contraction
CSA	Cross sectional area
$\dot{V}O_{2mx}$	Maximal oxygen consumption
V_{max}	Treadmill velocity max
CV	Coefficient of variation
RPE	Rate of perceived exertion
SD	Standard deviation
M	Mean
CL	Confidence Limits

CHAPTER ONE: INTRODUCTION

Exercise induced muscle damage (EIMD) occurs as a result of performing, novel, eccentric exercise (McHugh et al., 1999). This type of exercise occurs regularly in daily activities and is a beneficial training method for a number of populations (Howatson and van Someren, 2008). Athletes are regularly exposed to exercise, of which their bodies are not accustomed, which can increase susceptibility to EIMD (Versey, Halson and Dawson, 2013). For example, at championship level, athletes are required to perform at the highest standard daily (Leeder at al., 2012), often the time between events is not significant enough for athletes to recover sufficiently (Corbett et al., 2012). Consequently, athletes may experience the symptoms associated with EIMD such as: reduced muscle function, delayed onset muscle soreness (DOMS) and muscle swelling. The symptoms typically peak at 24-48 hours post eccentric exercise (Schoenfeld, 2012), therefore, it is important for athletes to consider recovery in their training programme. Recovery is defined as 'the return of the muscle to its pre-exercise state' (Tomlin and Wenger, 2000).

Previous literature has considered recovery techniques that include: the use of compression garments (Bieuzen et al., 2014; Hill et al., 2014), massage (Montgomery et al., 2008), ingestion of non-steroidal anti-inflammatory drugs (NSAID's) dietary supplements such as protein, carbohydrate and antioxidant supplementation and cryotherapy (Howatson and van Someren, 2008) in order to attenuate the deficit in performance post muscle damaging exercise. It is important to note that when considering an athlete's recovery, it is also necessary to consider how the intervention may impact on training adaptations, as it is important to achieve optimal adaptations throughout a training programme (Wilcock, 2006).

Water immersion is a popular strategy employed by athletes and coaches to attenuate the detrimental effects of EIMD on performance (Cook and Beaven, 2013). Water immersion is

commonly employed in the field as it can be performed at low cost and at relative ease within the field (Machado at al., 2015). However, protocols vary in: duration, depth and temperature (table 1) due to the lack of scientific evidence surrounding the mechanisms by which immersion protocols may enhance recovery (Vaile et al., 2008; White and Wells, 2013). Water immersion can be categorised as: cold water immersion (CWI), hot water immersion (HWI), thermo-neutral water immersion (TWI) or contrast water therapy (CWT) (Versey, Halson and Dawson, 2013).

The literature suggests that the beneficial effects of water immersion on recovery are a result of physiological changes stimulated by the temperature of the water and the action of hydrostatic pressure on the body (Versey, Halson and Dawson, 2013; Wilcock, Cronin and Hing, 2006). Specifically, the effects of cooling on the body are reported to be influential in the recovery process, with the magnitude of cooling related to the effect on markers of damage (White and Wells, 2013). However, the research into the effect of hydrostatic pressure on the body is somewhat equivocal, it is therefore important to consider the reasons for this variation. The literature has investigated the changes in hydrostatic pressure at different immersion depths by altering position of immersion from seated and standing (Leeder et al., 2015). However, it should be considered that changing depth of immersion alone may not stimulate significant pressure changes (Leeder et al., 2015). Once the mechanisms of water immersion have been considered, a protocol can be established that utilises the previous recommendations from the current literature surrounding duration and depth, in conjunction with research into the relative effects of hydrostatic pressure and water temperature to establish a more efficient immersion protocol that optimises recovery.

Research into the properties of water and the impact that these properties have on training, suggested that adding sodium chloride (NaCl) to water, to produce a saline solution, increases the hydrostatic pressure (Torres-Ronda and Alcazar, 2014). Nevertheless, to the best of the

authors knowledge there is no evidence to suggest that research to date has considered any other method of increasing hydrostatic pressure, other than changes in immersion depth. A saline immersion protocol is a novel immersion protocol that, in theory, is a strategy to further isolate the effects of increasing hydrostatic pressure and subsequently magnify the impact that hydrostatic pressure may have on recovery from EIMD.

Water immersion requires further research to better understand the relative effects of temperature and hydrostatic pressure on recovery from EIMD. Specifically, there is a need to develop further understanding of the effects of hydrostatic pressure, by conducting research into water immersion that isolates the effects of both hydrostatic pressure and temperature, as little research has previously reported this (Wilcock, 2006).

1.0.1 Aims of the Study

The primary aim of the study was to isolate the effects of temperature and hydrostatic pressure on the body by investigating the effects of CWI, TWI and thermo-neutral saline immersion (TSI) on markers of EIMD, to explore whether the physiological responses, stimulated by water immersion, are primarily a result of the actions of hydrostatic pressure or water temperature.

A secondary aim was to investigate the effect of a TSI protocol on the markers of EIMD.

CHAPTER TWO: LITERATURE REVIEW

This section will give an insight into the present literature surrounding EIMD and recovery via methods of water immersion. A literature search was conducted using sites that included; Web of Science, PubMed, Google scholar and the University of Bedfordshire online library resource. Key terms were searched in order to present relevant literature included: "Exercise induced muscle damage", "Recovery from exercise induced muscle damage", "Water immersion post exercise induced muscle damage". Primary sources, peer reviewed articles and meta-analysis were considered in this review.

2.0 An Overview of Exercise Induced Muscle Damage

2.0.1 Eccentric Exercise

Muscle contractions can be concentric, isometric or eccentric in nature (Ryschon et al., 1997). The literature reports that it is predominately eccentric contractions that result in exercise induced muscle damage (EIMD) (Howatson and van Someren, 2008). An eccentric contraction occurs when the force created by the muscle is less than that of the opposing force (McHugh et al., 1999), placing greater mechanical strain on the muscle (Springer and Clarkson, 2003). Conceicao et al. (2014) investigated the effect of varying the velocity of eccentric contractions (n = 9 Females (F), non-resistance trained, 24 ± 2 years; 57.8 ± 7.0 kg; 1.6 ± 0.5 m, 5 sets, 6 reps at slow velocity; 30°/ s or fast velocity; 210°/s, n = 10 F, non-resistance trained, 22 ± 4 years; 56.4 ± 6.0 kg; 1.6 ± 0.5 m). The authors concluded that the inflammatory response of the upper arm was not significantly altered between groups, suggesting that the velocity of the contraction may not have an effect on damage. However, it is important to consider that the study did not employ a repeated measures design, therefore, the individual response at each velocity cannot be determined.

Despite the increase in mechanical strain, eccentric contractions occur at a lower metabolic cost, therefore are beneficial to elderly and athletic populations (LaStayo, Reich and Urqhart et al., 1999; Hortobágyi et al., 2002). Additionally, eccentric contractions promote muscle hypertrophy, increase total strength and power and reduce the risk of injury (Bridgeman, McGuigan and Gill, 2015), therefore in order to optimise training there is a greater need to improve recovery from the symptoms of EIMD.

2.0.2 Repeated Bout Effect (RBE)

Although an initial bout of unaccustomed eccentric exercise may induce EIMD, there is evidence to suggest that following a subsequent bout of eccentric exercise, the magnitude of the damage is reduced (Chen et al., 2012). This is known as the repeated bout effect (RBE) (Nosaka et al., 2001; Clarkson and Hubbal, 2002; Chen and Nosaka, 2006; Bridgeman, McGuigan and Gill, 2015) and is present for up to 6 months post initial exercise bout (McHugh, 2003). However, this protective effect is restricted to the active muscles that have been exercised eccentrically (McHugh, 2003).

McHugh (2003) reported that the RBE is mediated by a combination of mechanical, neural and cellular mechanisms and adaptations. Subsequent to these adaptations, improved synchronization of motor unit firing reduces the mechanical stress placed on individual muscle fibres (Pierrynowski, Tudus and Plyley, 1987).

Mair et al. (1995) studied 22 male participants who were required to perform 70 eccentric contractions with the knee flexors and were asked to repeat this exercise 4 and 13 days post the initial exercise bout. The initial bout of eccentric exercise resulted in a decrement in muscle force and an increase in muscle soreness and CK ($p < 0.001$). However, participants who repeated the exercise bout at day 13 did not report an increase in muscle soreness, CK levels or any loss in muscle force compared to the first bout of eccentric exercise (Mair et al.,

1995). The results support the proposed explanation that increased efficiency of motor unit recruitment and utilisation may occur in a subsequent bout of eccentric exercise (McHugh, 1999). Nevertheless, it should also be considered that neural adaptations may not be solely responsible for the stimulation of the RBE.

2.1 Theoretical Explanations of Exercise Induced Muscle Damage

The body of literature surrounding EIMD purports theoretical metabolic and mechanical mechanisms of damage. This section will briefly address the proposed mechanisms. However, to examine in detail all of the proposed mechanisms of damage would be beyond the scope of this literature review. It should also be considered that the literature to date, remains inconclusive with regard to the precise mechanism of damage.

2.1.1 Metabolic Mechanisms

Metabolic mechanisms, described within the literature account for the changes in cellular homeostasis (Howatson and van Someren, 2008). It is reported that changes in cellular homeostasis are a result of ischemia or a hypoxic environment created when exhaustive exercise is performed (Armstrong, Warren and Warren, 1991). Little research explores the metabolic actions that contribute to the symptoms of EIMD and although a number of reviews highlight potential mechanisms, much of the literature draws hypothetical conclusions and proposed mechanisms of damage (Armstrong, Warren and Warren, 1991). Until more conclusive evidence is available, it should be considered that metabolic mechanisms are not solely responsible for damage that occurs as a result of eccentric exercise.

2.1.2 Mechanical Mechanisms

Mechanical mechanisms of damage refer to the structural changes that result in the symptoms of damage (Proske et al., 2001). Eccentric contractions place high strain on low fibre

recruitment (Friden and Lieber, 1992), causing disruption to the sarcolemma, cytoskeletal damage, swelling of the sarcotubular system and distortion to the contractile components of the myofibres (Friden and Lieber, 1992).

Sarcomere Inhomogeneity

Morgan (1990) proposed the theory of 'popping' sarcomere hypothesis as a likely explanation for the reduction in neuromuscular (NM) function following EIMD (Byrne, Twist and Eston, 2004). High stress placed on reduced low fibre recruitment, results in overextended sarcomeres, subsequently, non-uniform lengthening of the sarcomere occurs (Byrne, Twist and Eston, 2004). This causes a shift in the length tension relationship and a decrease in strength (Byrne, Twist and Eston, 2004). The physical stress of tearing and subsequent alteration of calcium ion homeostasis results in the symptoms of EIMD (Morgan and Proske, 2004). However, sarcomere 'popping' was not observed by Telley et al. (2006) who examined the effect of actively stretching half sarcomeres by 15-20% of the resting length, and observed that although sarcomeres lengthened non-uniformly, lengthening beyond myofilament overlap did not occur. However, Telley et al. (2006) conducted research on an animal population. The muscle stretching occurred within a laboratory setting using rabbits, therefore, does not replicate the strain of an athlete's workload or biological differences between athletes and animals. This should be carefully considered before this theory is applied to an athlete of a trained state.

Excitation Contraction Coupling

The E-C coupling system was described by Warren et al. (2001) as the initial release of acytlecholine which results in the production of an action potential, this travels through the muscle fibre via the tubule. Although the precise mechanisms of E-C uncoupling remain unclear. Franzini-Armstrong and Jorgensen (1994) proposed that E-C coupling depends on the communication between the junctional domains of the sarcoplasmic reticulum,

sarcolemma tubules and transverse tubules. The proposed pathway suggests that the depolarization of the T-tubular membrane stimulates a change in the voltage sensors (Franzini-Armstrong and Jorgensen, 1994; Warren et al., 2001), stimulating the sarcoplasmic reticulum release channel to release Ca^{2+} into the cytosol, enabling Ca^{2+} to bind to troponin for cross bridge cycling to occur (Warren et al., 2001). E-C uncoupling occurs during eccentric exercise as a result of the elevation of the levels of free Ca^{2+} in the cytoplasm of the muscle fibre (Proske and Morgan, 2001). The elevation of Ca^{2+} beyond normal levels disrupts the E-C coupling process, whereby disruption at the triad junction occurs, inhibiting the interaction with the sarcoplasmic reticulum (SR) Ca^{2+} release channel (Lamb, 2009). An influx of Ca^{2+} activate proteolytic pathways leading to structural damage, specifically, the degradation of muscle myofibre membrane and contractile proteins (Tee, Bosch and Lambert, 2007) therefore, E-C uncoupling is considered a mechanical mechanism of damage.

Discrepancies within the literature regarding the primary mechanisms of damage remain, however, this may be a result of differences in exercise protocols. Therefore, it may be explained that different exercise modalities stimulate a differing mechanistic actions (Tee, Bosch and Lambert, 2007), additionally it could be suggested that no single mechanism is dominant in the occurrence of the symptoms of EIMD.

The symptoms of EIMD include: increased CK activity, increased perceptions of muscle soreness, delayed onset muscle soreness (DOMS), a deficit in force production and muscle swelling (McHugh et al., 1999; Plattner, Lambert and Baumeister, 2012; Chen et al, 2012). However, the magnitude of the damage may be altered by factors such as: age, gender, muscle length and the number of muscle contractions (Nogueira et al., 2013). Moreover,

recent suggestion that training status may impact response to muscle damage and recovery protocols should be considered.

2.1.3 Effect of Training Status on Recovery from Exercise Induced Muscle Damage

Endurance trained and resistance trained individuals present with physiological differences. Resistance training promotes greater muscle fibre hypertrophy over endurance training programmes (Leveritt et al., 1999). A resistance training programme incorporates a greater volume of eccentric components, due to the positive effects associated with eccentric exercise (Howatson et al., 2012). Subsequently, these individuals may experience an attenuation of the effects of muscle damage, due partly to the effects of the RBE (section 2.0.2). Endurance training stimulates adaptation to neuromuscular, pulmonary and cardiovascular systems (Jones and Carter, 2000). Due to the difference in adaptations as a result of training, it is reasonable to suggest that there will be differing responses to EIMD and recovery. Therefore, it is critical that training status is considered before implementing a recovery protocol. Therefore, novel recovery techniques require further research across populations of differing training status' before they can be recommended within athletic populations.

2.1.4 Hormesis

Hormesis refers to the stimulation of damage, however, this damage is followed by a beneficial adaptive process (Peake et al., 2015). Research has suggested an 'optimum' level of damage, whereby, the adaptation response, stimulated by damage, is not inhibited too much by a recovery intervention. Recovery interventions should consider this 'optimum' level of damage in order to achieve the greatest levels of adaptation, combined with the attenuation of the detrimental effects of damage on performance (Peake et al., 2015; Howatson, Leeder and van Someren, 2016). Therefore, when employing a recovery method during a training season, it is necessary to consider a holistic view that enables an athlete to continue training without the inhibiting adaptation via restriction of inflammation.

2.2 Methodology of Measuring Markers of Exercise Induced Muscle Damage

Many symptoms and markers of EIMD are reported within the literature, however, not one marker has yet been established as a gold standard measure of EIMD (Paulson et al., 2012). This presents a challenge to identify and accurately assess the effects of EIMD on performance.

2.2.1 Inflammatory Markers

EIMD stimulates an inflammatory response that transfers fluid and cells to the site of damage (Clarkson and Sayers, 1999). Eccentric exercise stimulates an influx of inflammatory cells (Tidball, 2004) and leukocyte infiltration to occur at the sight of trauma (Malm, 2001), resulting in a change in the concentration of circulating cytokines (Hirose et al., 2004). The magnitude of this disturbance is relative to the duration and intensity of the exercise (Clarkson and Sayers, 1999).

Inflammation is a necessary process that is required in order to stimulate muscle repair, remodelling and regeneration (Tidball, 2004). Eccentric exercise damages the integrity of the cell, increasing cell and lymph permeability at the site of trauma (Proske and Morgan, 2001), subsequently increasing blood flow to the area before regeneration and fibrosis can occur (Prisk and Huard, 2003). This in turn induces the formation of oedema that initially amplifies the pain that an individual experiences (Clarkson and Hubbal, 2002) and causes a reduction in range of motion (ROM).

Inflammation is reported to peak 1-5 days post damaging exercise (Pederson, 2001; Chen et al., 2012), where neutrophils, lymphocytes and monocytes are activated by cytokines circulating at the sight of trauma (Pederson, 2001). Following which, macrophages populate the site, whereby the clearance of cellular debris and phagocytosis occurs (Prisk and Huard, 2003). Macrophage infiltration may also lead to further tissue damage as a result of the

spillage of degradative enzymes (Lapointe et al., 2012, cited in Prisk and Huard, 2003). Therefore, reducing inflammation post exercise may attenuate the degeneration of muscle, however, it should be carefully considered that reducing inflammation may also reduce the repair and regeneration (figure 1) of the muscle (Prisk and Huard, 2003), having a subsequent effect on hormesis as it has been noted that inflammation is part of the adaptive response that enhances the strengthens muscle structures (Barnett, 2006).

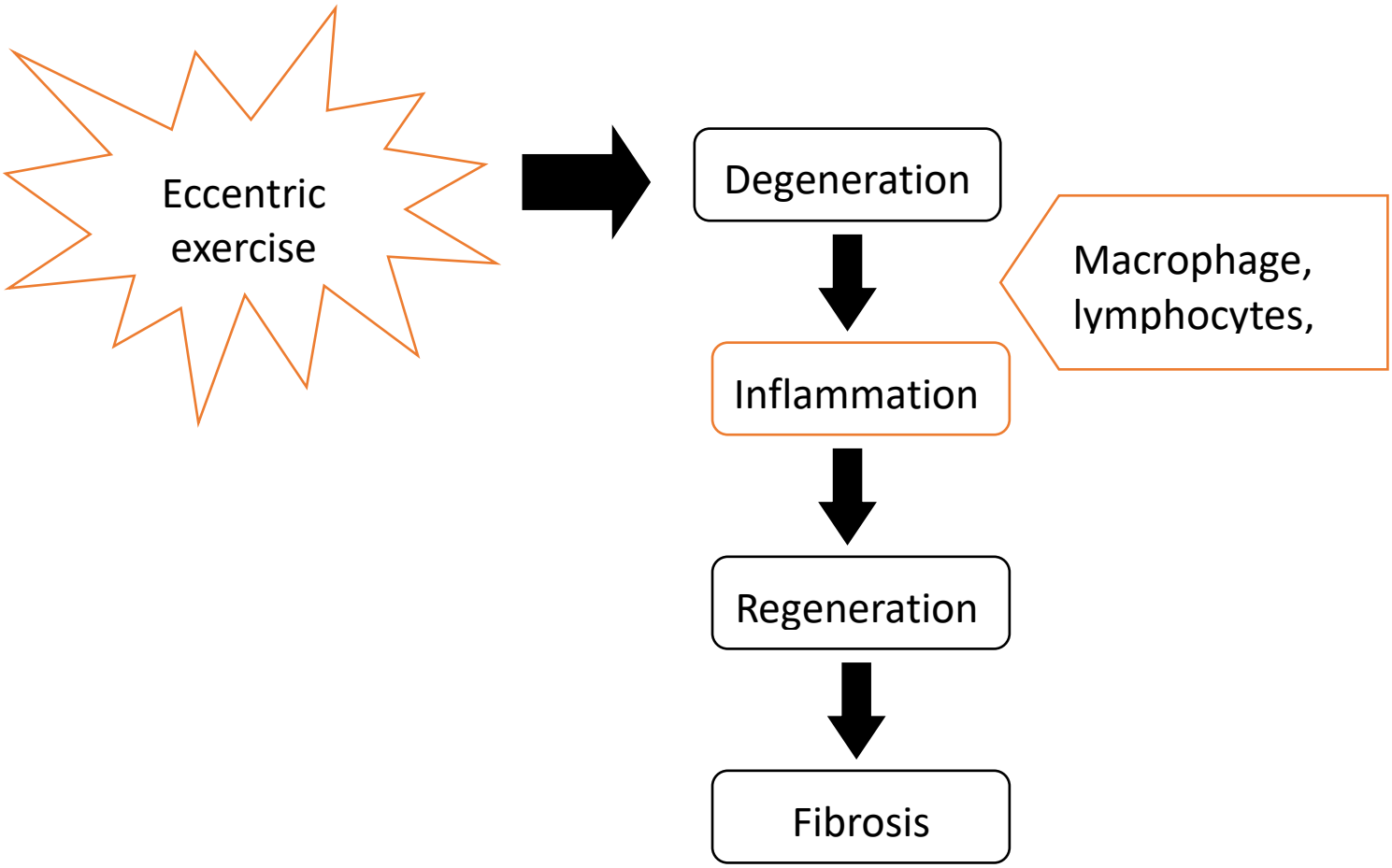


Figure 1. Adapted from Prisk and Huard (2003) demonstrates the repair and regeneration of the muscle post eccentric exercise and the importance of inflammation in the process of repair.

Limb circumference is widely reported within the literature pre and post muscle damaging exercise. A significant increase in limb circumference post EIMD was reported in several studies when no intervention was applied (Eston and Peters, 1999; Murayama et al., 2000; Vaile, Gill and Dawson, 2008; Chen et al., 2012). However, not all studies report a significant increase in limb circumference post EIMD. Crystal et al. (2013) used a muscle damaging protocol of a 40 minute downhill run at -10% gradient at 60% of participants VO_{2peak}, which induced sufficient damage to significantly increase perceptions of pain ($p < 0.001$) and significant deficits in peak torque ($p < 0.001$), however, no significant change in limb circumference occurred from pre muscle damaging exercise to post ($p > 0.05$). Analysis of limb circumference provides little insight into the mechanistic actions that account for swelling such as changes in blood cytokine concentrations. Moreover, there are methodological limitations to consider with measures of limb circumference. This measure may be altered by human error, therefore, it is necessary to perform measures of reliability and familiarisation sessions to account for this. Researchers have minimised the effect of error by marking with semi permanent marker, at the point of measurement, in order to obtain the same measurement at each time point (Goodall and Howatson, 2008). Nevertheless, when considering the limitations associated with analysis of limb circumference, it may be necessary to report additional markers of damage.

Analysis of cross sectional area (CSA) of the muscles via an ultra-sound scan is a marker that, to the best of the authors knowledge, is not reported within the literature as a marker of EIMD. However, analysis of CSA could be utilised, as a non-invasive method of assessing damage at isolated muscles. However, despite the benefit of such direct analysis, there are issues of reliability of the measure to consider. One method of analysing ultra-sound images relies on using computer programmes, such as Photoshop or ImageJ (Lima, Matta and Oliveira, 2012) to establish CSA. Some analysis software, including ImageJ, requires the

conversion of files into different formats, thus altering the pixels causing the measurement to be invalid. Therefore, CSA analysis should be conducted on software that can measure DICOM images, such as Photoshop. Additionally, the process of analysis requires manual tracking of the border of the muscle for this measure to be reliable (Lima, Matta and Oliveira, 2012). Previous analysis of the reliability of CSA of rectus femoris has reported a coefficient of variation (CV) of 8.53 % at 15cm above the patella and 8.9 % at 50% of the thigh length, suggesting that the measure is reliable (Lima, Matta and Oliveira, 2012). However, there were differences in CSA when obtaining a measure between the two sites. To report CSA via ultra sound scan, the point of measurement should be consistent across time points and reliability of the measure should be assessed prior to data collection.

2.2.2 Blood Markers

Blood markers of muscle damage are also reported within the literature as indirect markers of damage, specifically, CK activity is monitored pre and post muscle damage (Leeder et al., 2015; Glasgow, Ferris and Bleakley, 2014; Pointon et al., 2011; Goodall and Howatson, 2008; Halson et al., 2008) and to a lesser extent the effect of EIMD and recovery protocols on lactate dehydrogenase (BLa) (Pointon and Duffield, 2012; Vaile, O' Hagan and Stefanovic, 2011; Gleeson et al, 1998).

CK is an enzyme located in the cytosol and mitochondria of tissue (Baird et al., 2012). Myofibrillar CK (CK-MM) is an important component that circulates in active muscle. It is the CK-MM isoenzyme that is stimulated in skeletal muscle damage (Baird et al., 2012).

The literature remains equivocal with regard to the reliability of reporting CK activity post muscle damaging exercise (Baird et al., 2012). CK-MM is located in the muscle cell, therefore, an efflux of CK that occurs in the blood is representative of muscle damage or loss of cell integrity (Baird et al., 2012). Levels of CK peak 24 - 48 h post muscle damaging

exercise and have reportedly occurred alongside the greatest perceptions of soreness (Reilly and Ekblom, 2005). This efflux is linked with the subsequent strength deficits associated with EIMD (Sellwood et al., 2007). Resting CK levels can vary from 35-175 IU. L^{-1} in the general population (Gagliano et al., 2009). Additionally, when an individual experiences clinically diagnosed muscle damage, CK levels can reach 10,000- 200,000 IU. L^{-1}, although, damage is considered to be of significant disturbance when CK levels reach > 5,000 IU. L^{-1} (Huerta-Alardin, Varon and Marik, 2005). The vast variations in resting levels of CK amongst different populations, combined with the broad values that are considered in a clinical setting demonstrate a weakness of CK as a marker of EIMD. Stupka et al. (2001) concluded that CK as a marker of damage should be interpreted with caution due to the weak representation of actual skeletal damage that CK indicates. Moreover, research has suggested issues of reliability, when using a Reflotron for blood analysis (Selmer, Foss and Lund-Larsen, 1990). When analysing vast quantities (> 700) of samples, coefficient of variation (CV) increased to 5.2%, compared to, analysing smaller samples (≤ 500) that reported a CV of 1.8% for analysis of cholesterol (Selmer, Foss and Lund-Larsen, 1990). Horder et al. (2001), analysed CK using a Reflotron and reported a median CV of 3.1%, however, it should be considered this study was published 15 years ago. Improvements in technology may have addressed reliability of the current equipment used to analyse CK, and should be investigated in future research, as to the best of the authors knowledge, there are no current data to support this assertion. The literature reports the use of a Reflotron to analyse CK as an easy to operate, quick method of assessing CK (Glasgow, Ferris and Bleakley, 2014; Goodall and Howatson, 2008; Horder et al., 2001). Additionally, previous analysis of CK has been conducted after log transforming the data set, or reporting data as a percentage of baseline values, in order to aid the interpretation of results (Goodall and Howatson, 2008). Therefore, it can be concluded that despite equivocal reports of CK analysis, it is an important factor to consider to obtain a

holistic assessment of muscle damage and recovery, nevertheless, these findings should be interpreted with caution.

2.2.3 Performance Markers

Eccentric exercise inhibits neuromuscular (NM) function and results in a prolonged reduction of muscle function (Byrne, Twist and Eston, 2004). Increased soreness occurs due to micro trauma in the muscle fibre making an individual more susceptible to muscle spasm and increasing pain (White and Wells, 2013). Consequently, a reduction of maximal force production (Plattner, Lambert and Baumeister, 2014) occurs. A reduction in NM function occurs when performing movements that involve high occurrence of the stretch shortening cycle (SSC), whereby the active muscle contracts eccentrically followed by a concentric contraction, that commonly occur in actions such as downhill running, resistance training and plyometrics (Byrne, Twist and Eston, 2004). However, the exact mechanisms by which these reductions in NM function occur post eccentric exercise protocols are yet to be established (Clarkson and Hubal, 2002). For optimal force production to occur, physiological levels of reactive oxygen species (ROS) are necessary, however, if these levels are elevated beyond the normal physiological level, muscular dysfunction occurs and subsequently muscle fatigue (Powers and Jackson, 2008). To attenuate the deficit in performance it is necessary to further understand the mechanisms that cause this reduction.

Performance markers are widely reported and considered a reliable indirect markers of damage (Hyldahl and Hubal, 2014; Corbett et al., 2012; Goodall and Howatson, 2008; Hirose et al., 2004). Multiple methods have been employed to assess and monitor changes in muscle force production pre and post muscle damaging exercise, which include, maximal voluntary isometric contraction (MVIC) (Hyldahl and Hubal, 2014; Corbett et al., 2012; Goodall and Howatson, 2008; Hirose et al., 2004), countermovement jumps (CMJ) (Crystal et al., 2013;

Leeder et al., 2015), vertical jump (Bailey et al., 2007) squat jumps and sprint performance (Ascenao et al., 2011; Hill et al., 2014).

Reduction of maximal force production has a significant impact on athletic performance; Clarkson et al. (2002) reported strength deficits of 50-65% after a bout of high force eccentric exercise (maximal eccentric contractions). The literature reports that a strength deficit may be present for up to 10 days post muscle damaging exercise (Clarkson et al., 1992) and peaks 2-3 days post (Clarkson and Sayers, 1999). Leeder et al. (2015) observed significant reductions in maximal strength via assessment of CMJ ($p < 0.001$) and MVIC ($p = 0.009$) post muscle damaging exercise (Loughborough intermittent sprint test, 5 x 15 minutes at varying intensity ~55% - 95% $\dot{V}O_{2max}$). Strength deficits peaked 24 hours post exercise and lasted up to 72 hours post (Leeder et al., 2015). Similarly, Pointon et al. (2011) reported a significant reduction in MVIC ($p = 0.01$) post muscle damaging exercise (6 x 25 maximal concentric/eccentric knee extensor contractions). Strength deficits were reported to last the duration of the 48 hour recovery assessment period (Pointon et al., 2011). Finally, a reduction in MVIC was reported post muscle damaging exercise (100 drop jumps; 5 x 20 repetitions separated by 2 minutes rest) (Howatson, Goodall and van Someren, 2009). A reduction of ~89% in MVIC was reported post exercise bout, with strength deficits present up to 96 hours post exercise (Howatson, Goodall and van Someren, 2009). The results from the studies presented, demonstrate that MVIC and CMJ are commonly used assessment methods in EIMD literature. However, it should be considered that a learning effect may be present when analysing performance markers. Meldrum et al. (2003) reported greatest variation and the presence of a learning effect of MVIC in individuals who were not familiar with the equipment used. Sufficient familiarisation is necessary to counteract this effect.

2.2.4 Delayed Onset Muscle Soreness

Delayed onset muscle soreness (DOMS) refers to the perceived pain and stiffness that occurs post EIMD (Armstrong, 1984). Initial pain is present at the muscular tendon junction, however over time spreads throughout the muscle (Lieber and Friden, 2002) and can last several days post exercise (Nosaka et al., 2001; Leeder et al, 2011). Symptoms typically peak approximately 24-48 hours post exercise (Sellwood et al., 2007; Connolly et al, 2003; Gleeson et al., 1998) and subside within 96 hours when a recovery intervention is not applied (Friden and Lieber, 2002). A number of possible mechanisms are reported in the literature and include the action of: lactic acid, muscle spasm, connective tissue damage and inflammation (Cheung, Hume and Maxwell, 2003).

Early literature suggested that DOMS occurs as a result of stretch induced muscle injury that leads to the excessive strain on connective tissue (Hough, 1902) subsequently resulting in the disruption of muscle fibres and the contractile properties of the fibre. Further soreness is a result of the subsequent inflammatory response (Umbel, 2009) which occurs due to; vasodilatation, increased capillary permeability and an increase in extracellular protein (Burt et al., 2014). However, fibre damage is not solely responsible for the increase in perceptions of pain, this is known from examination of the muscle fibres of clinical cases of Duchenne muscular dystrophy (Friden and Lieber, 2002). In a review of the mechanical basis of DOMS, Friden and Lieber (2002) reported that examination of the muscle biopsies of patients with Duchenne muscular dystrophy demonstrated significant disruption of the muscle myofibre, however, the patients did not report to be in any pain. Therefore, perceptions of pain cannot be a primary response of myofibre damage, more likely a result of the secondary phase associated with damage (Friden and Lieber, 2002).

Delayed onset muscle soreness (DOMS) is one of the most commonly reported injuries in sport following EIMD (Cheung, Hume and Maxwell, 2003) and results in alterations of:

running economy, caused by changes in lower limb kinematics, blood lactate and ratings of perceived exertion (Burt et al., 2014). These factors all contribute to a reduction in performance. A visual analogue scale (VAS) is often used to measure DOMS (Burt et al, 2014), as perceptions of pain are an important marker to obtain in order to understand the effectiveness of an intervention as a recovery aid (Vaile, Gill and Blazevich, 2007; Howatson and van Someren, 2009; Leeder et al., 2015; Ascensao et al., 2011; Corbett et al., 2012). Zusman (1986) reported the use of an absolute visual analogue scale to be the best 'pencil and paper method' to obtain a measure of pain intensity. This scale uses a line with conflicting descriptions at either end, such as: 'no pain' at one end of the line and 'worst possible pain' at the other end. This offers a continuous scale for participants, compared to a quantifying pain via a numbering system that have previously been reported (Zusman, 1986).

Nevertheless, assessment of pain via a VAS post EIMD is a subjective measure that poses difficulty to standardise. Attempts to control the assessment of pain are emerging in the literature. Leeder et al. (2015), Howatson, Goodall and van Someren, (2009) and Burt et al. (2014) reported perceptions of pain post EIMD using a VAS scale, however, participants were asked to place their hands on their hips and squat to 90° at which point they were asked to mark their perception of pain on a 0-200 mm scale. Another method that has been utilised to standardise the assessment of pain is demonstrated by Close et al. (2006) who used pressure algometry to apply a set amount of pressure at the site of damage to each participant. Utilising such protocols provides an assessment of the pain at specific muscle groups by isolating the perception of pain to specific muscle groups. Nevertheless, it remains a subjective measure of pain due to large individual variation in pain thresholds (Cleak and Eston, 1992).

2.2.5 Summary of the Methodology

To analyse the effect of an intervention on recovery, it is necessary to assess inflammatory, blood, performance and perceptual markers of damage. Generally, the literature is successful in evaluating a holistic effect of recovery interventions on EIMD. Issues regarding reliability of markers of swelling remain throughout studies that report measures likely to be influenced by researcher error. Additionally, perceptual markers will remain susceptible to individual interpretation. Sufficient familiarisation of all outcomes measures should be performed to minimise the occurrence of error and to standardise performance measures. Nevertheless, few studies report the use of the same experimental protocols to assess recovery, therefore the transfer of results and conclusions between studies, may, at times, be compromised.

2.3 Recovery from Exercise Induced Muscle Damage

To attenuate the symptoms of EIMD it is necessary to apply an intervention that targets the symptoms of damage described in the sections above. Many recovery techniques are reported within the literature (Leeder et al., 2015; Pasiakos, Lieberman and McLellan, 2014; Bieuzen et al., 2014; Hill et al., 2013; Versey, Halson and Dawson, 2013; Best, Gharaibeh and Huard, 2012; McGuinley, Shafat and Donnelly, 2009; Howatson and van Someren, 2008; Bailey et al., 2007; Barnett, 2006; Cochrane, 2004). Despite the quantity of literature investigating the use of various interventions, discrepancies regarding the most effective method of attenuating the symptoms of EIMD remain apparent, and to examine all of these modalities would be beyond the scope of this literature review. This section will examine the studies that have utilised water immersion to assess recovery from EIMD.

Water immersion is a recovery technique that is becoming increasingly popular amongst athletic populations, to attenuate the detrimental effects of EIMD (Howatson et al., 2009). Immersion strategies induce physiological responses such as: increased heart rate (HR), a

reduction in swelling, enhanced clearance of CK and analgesic effects amongst others to attenuate the response to EIMD, however the research regarding the most effective immersion protocol remains equivocal (Vaile et al, 2008). The consensus is that the water temperature and the actions of hydrostatic pressure on the body stimulate the responses that aid recovery (Wilcock et al., 2006). However, vast variation in protocols of water immersion employed (table 1), results in variation of physiological response and effectiveness of the intervention. In addition to the mode by which damage is implemented, the depth, temperature and duration of an immersion protocol should be carefully considered.

Table 1. A summary of studies demonstrating the variation in immersion durations, temperature and depths employed within the present literature surrounding CWI and recovery from exercise induced muscle damage

Author	Participants	Exercise Protocol	Intervention	Timing of intervention	Outcome measures	Results
Ascensão et al. (2011)	20 (M) Junior national league footballers: 18.1+1.8 yrs, 181.6+0.4 cm, 68.4+3.8 kg	Friendly football match	CWI: 10 min 10˚C, iliac crest, seated TWI: 10 min 35˚C, iliac crest, seated	Immediately post	Sprints, CMJ, MVIC Quadriceps, CK, RPE, perceived soreness	CWI: ↑ CK compared to TWI, ↑ MVIC compared to TWI, ↓ soreness in CWI group
Bosak et al. (2009)	12 Trained runners (9 M, 3 F) Characteristics not reported	5 km running trial, repeated 24 hours later	CWI: 12 min 16˚C CON: Passive recovery, posture and depth not reported	Immediately post	Running performance, HR, RPE, perceived fatigue, muscle soreness	CWI: ↓ 5km running time trial, average HR and RPE post exercise compared to CON
Eston and Peters (1999)	15 (F) university students: 22.0 ± 2.0 yrs	8 x 5 max concentric & eccentric isokinetic contractions (Elbow flexors)	CWI: 15 min 15˚C, arm immersed, CON: No treatment	Post exercise, repeated every 12 h for 3 days	MVIC elbow flexors, arm girth, CK, muscle soreness, relaxed arm angle	CWI: ↑ relaxed arm angle (↑ ROM), ↓ CK 48 and 72 h post

Author	Participants	Exercise Protocol	Intervention	Timing of intervention	Outcome measures	Results
Howatson et al. (2009)	16 (M) Recreationally Active: 23 ± 3 yrs; 1.81 ± 0.04 m; 82.7 ± 10.7 kg	Drop Jumps (5 x 20)	CWI: 12 min 15 ± 1˚C, illiac crest, posture not reported CON: 12 min seated rest	immediately post, 24, 48 and 72 h post exercise	CK, muscle soreness, mid-thigh girth, MVIC of knee extensors	CWI: no significant effect on outcome measures compared to CON
Jakeman, Macrea and Eston (2009)	18 (F) Recreationally active: 19 ± 1 yrs: 1.66 ± 0.55 m; 63.7 ± 10 kg	CMJ (10 x 10)	CWI: 10 min 10 ± 1˚C, iliac crest, seated immersion CON: No treatment	Within 10 min post exercise	CK, Limb soreness, maximal isokinetic concentric strength of quadriceps	CWI: no significant effect on outcome measures compared to CON
Peiffer et al. (2009)	10 (M) Well trained cyclists: 27±7 yrs; 181±6cm; 77.9±6.6kg	90 min cycling at 80% ventilatory threshold power, 16 min TT at 32.2˚C	CWI 20 min 14˚C to mid sternal, seated immersion, CON: 20 min seated rest in 24˚ C	25 minutes post exercise	Knee extensor MVIC, femoral vein diameter, skin temp, rectal temp	CWI: ↓ MVIC, skin temp, rectal temp and femoral vein diameter compared to CON

Author	Participants	Exercise Protocol	Intervention	Timing of Intervention	Outcome measures	Results
Stanley et al. (2012)	18 endurance trained cyclists: 27 ±7 yrs; 75.2 ± 9.0 kg; 1.82 ± 0.06 m	60 min, high intensity cycling, (8 x 4min at 80% peak power)	CWI: 5 min 14˚C to shoulder level immersion CON: 10 min seated rest	20 minutes post exercise	Work-based cycling time trial (15min, 75% peak power), HR, perceived fatigue, muscle soreness (legs), mental & physical recovery	CWI: ↑ perceived recovery compared to CON. ↓ muscle soreness compared to CON.
Vaile et al. (2008)	12 strength trained (M): 32.2 ± 4.3yrs, 176.6 ± 4.5 cm, 68.8 ± 7.2 kg	Eccentric leg press (5 x 10 at 120% 1RM, 2 x 10 at 100% 1RM)	CWI: 14 min 15 ˚C, shoulder level, standing immersion CON: 14 min seated rest	Immediately post exercise repeated 24, 48 and 72 h post	Squat MVIC , loaded squat jump max power, thigh circ., muscle soreness, CK	CWI: ↓squat MVIC compared to CON, ↓ thigh circumference & CK 24 &72 h post compared to CON

Author	Participants	Exercise Protocol	Intervention	Timing of intervention	Outcome measures	Results
Vaile, O' Hagan and Stefanovic (2011)	10 endurance trained (M): 34 ± 5 years; 180.1 ± 5.2 cm; 78.6 ± 7.3 kg	15 min cycling at 75 % peak power, followed by 15 min TT, repeated 60 min post	CWI: 15 minutes, 15 ˚C, shoulder level, standing immersion ACT: 15 min cycling at 40% peak power output	5 minutes post exercise	Cycling TT total work, rectal temp, Bla, HR, leg and forearm blood flow	CWI: ↑ TT time and Bla compared to ACT. ↓ Rectal temp and blood flow versus ACT.

M: Males, F: Females, CWI: cold water immersion, TWI: thermo-neutral immersion, CON: control, CMJ: countermovement jumps, MVIC: maximal voluntary isometric contraction, CK: creatine kinase, RPE: rate of perceived exertion, ↑: indicates significant increase, ↓: indicates significant decrease, HR: heart rate, ROM: range of motion, TT: time trial, RM: rep max.

2.3.1 Cold Water Immersion

CWI acts by decreasing skin temperature and muscle temperature (Versey, Halson and Dawson, 2013). By reducing core temperature, thermoregulatory fatigue is reduced (Peiffer et al., 2009). Additionally, the reduction in temperature stimulates vasoconstriction via the cutaneous receptors, stimulating the sympathetic fibres (Cochrane, 2004), whereby peripheral resistance and blood pressure increase and blood is redistributed from the peripheral to the central cavity to maintain core temperature (Wilcock et al., 2006). Vasoconstriction reduces blood flow, reducing inflammation and attenuating the production of metabolites, thus reducing the build-up of metabolites at the site of the injury (Cochrane, 2004). Cold application also attenuates the permeability of lymph and capillary walls, accounting for the reduction in CK efflux (Eston and Peters, 1999).

CWI is reported to increase parasympathetic nervous system activation post muscle damaging exercise (Stanley et al., 2011). Moreover, the applications of CWI can result in a reduction of contractile velocity, stimulating a reduction in muscle spasticity and pain, however, this may be detrimental to muscle force generation (Wilcock et al., 2006). Despite the reports of these physiological responses within in the literature, the effectiveness of CWI on recovery from EIMD remains equivocal.

Research conducted into the effect of CWI on recovery from EIMD demonstrated no significant effect of a CWI protocol on markers of knee extensor MVIC, muscle soreness, mid-thigh girth or measures of CK when compared to a control group (Howatson et al., 2009; Jakeman, Macrae and Eston., 2009, table 1). However, further investigation (Vaile et al., 2008, experiment 1) reported that CWI is effective in increasing squat MVIC and peak power production compared to a control group at 48 h ($p = 0.01$) and 72 h ($p = 0.03$) suggesting no detrimental effect of CWI on contractile velocity. A significant reduction in thigh

circumference and CK at 24 ($p < 0.03$, $p = 0.03$) and 72 h post exercise ($p < 0.03$, $p = 0.04$) respectively, was also reported compared to a control group. Additionally, Vaile, O'Hagan and Stefanovic (2011) reported no change in cycling time trial performance in the CWI group compared to a significant decline in the active recovery group. CWI group also reported decreased rectal temperature ($p < 0.05$) and reduced blood flow ($p < 0.05$) until the end of the second exercise bout. However increased blood lactate levels were reported in the CWI group (4.5 ± 1.2 mM) compared to active recovery group (2.3 ± 0.8 mM) ($p < 0.05$).

Furthermore, Bailey et al. (2007) studied the effects of CWI recovery intervention (10 minutes at 10˚C agitated water immersed to the iliac crest) compared to a control group (10 minutes seated rest) post muscle damaging exercise (n = 20 males, 22 ± 3 years; 180.0 ± 0.05 cm; 83.7 ± 11.9 kg). The results stated CWI was successful in attenuating the decrement in knee flexion MVIC at 24 h post (12 ± 4 %) and 48 h (3 ± 3 %) and reduced perceptions of soreness up to 48 h post ($p < 0.05$), compared to a control group at 24 (21 ± 5 %) and 48 h (14 ± 5 %) ($p < 0.05$). Using the same immersion protocol, Ascensao et al. (2011) reported significant reductions in pain and a significant attenuation in the decrement of quadriceps MVIC at 24 h compared to a TWI group ($p < 0.05$) (see table 1). Nevertheless, both Bailey et al. (2007) and Ascensao et al. (2011) used a football match to induce damage, this may not reflect the same magnitude of damage compared to exercise protocols performed in a laboratory.

Although Bailey et al. (2007) and Ascensao et al. (2011) employed methods of inducing damage that are ecologically valid, it is possible that each individual would experience varying degrees of damage dependent upon the individual work rate, unlike the standardised protocols employed by Howatson et al. (2009) and Jakeman, Macrae and Eston, (2009). Furthermore, Leeder et al. (2015) reported no effect of CWI (14 min at 14 °C, seated and

standing immersion) following EIMD (Loughborough Intermittent Shuttle Test, LIST), which contradicts the results of Bailey et al. (2007). This contradiction may be accounted for by the variation in methods and immersion protocols employed. The heterogeneity in methods employed present challenges when comparing studies and interpreting the literature. Therefore, this should be considered when comparing the success of a CWI protocols across different EIMD protocols.

Explanations for the lack of significance reported in the literature may be accounted for by immersion depth to be discussed further in section 2.3.3. The associated effects of depth may explain the lack of significance reported by Howatson et al. (2009) and Jakeman, Macrea and Eston (2009) as these studies employed immersion protocols to the iliac crest. However, the protocol employed by Vaile, O'Hagan and Stefanovic (2011) also immersed participants to the iliac crest, but a positive effect of CWI on recovery was reported. In this instance, CWI was performed at 10˚ C unlike the protocol implemented by Howatson et al. (2009) (CWI at 15˚C). Initial consideration of these findings may suggest that the effectiveness of a CWI protocol is temperature dependent. Eston and Peters (1999) confirmed that the rate of decrement of the sensory and motor nerve conduction velocity is proportional to the tissue temperature and is continuous until nerve tissue is cooled sufficiently to 10-15˚C. Nevertheless, it is important to consider that cooling of the muscle may also have detrimental effects. White and Wells (2013) reported that cooling of the muscle can significantly reduce the rates of tension development in the non-damaged skeletal muscle, suggesting that cooling may impact the rate of excitation-contraction coupling. Furthermore, the literature has previously reported that CWI may inhibit training adaptation (Versey, Halson and Dawson, 2013). Nevertheless, more recent literature has contradicted these reports, and has suggested that CWI can promote the up-regulation of PGC-1α expression, thus stimulating adaptation

(Ishan, 2014; Ishan, 2015; Joo et al., 2016). The conflicting literature should be carefully considered when applying a CWI to assist recovery.

2.3.2 Thermo-neutral Water Immersion

Thermo-neutral water immersion (TWI) is reported in the literature as water immersion in temperatures of 21-35°C (Torres-Ronda and Alcazar, 2014) as water of this temperature does not alter core body temperature (Versey, Halson and Dawson, 2013).

However, there is little evidence to suggest that TWI is effective as a recovery aid. Ascensao et al. (2010) examined the effect of CWI and TWI protocols post muscle damaging exercise following a soccer match. The findings concluded that TWI (10 minutes at 35°C) had little beneficial effect on recovery, with significant reductions in peak quadriceps strength at 24 and 48 h ($p < 0.05$), CMJ at 24 and 48 h ($p < 0.05$) and squat jumps at 24 h post ($p < 0.05$) (Ascensao et al., 2010). Cochrane (2004) stated that for physiological responses to occur, muscle temperature must be sufficiently heated or cooled. As a direct result of this, any beneficial effects reported are considered a result of hydrostatic pressure on the body (Al Haddad et al., 2010). Analysis of the literature has presented varying success of water immersion protocols, this variation demonstrates a need for greater understanding into the action and importance of temperature and hydrostatic pressure.

2.3.3 Hydrostatic Pressure

Water immersion is reported within the literature to produce a 'squeezing' effect on the body, this is a result of hydrostatic pressure (Wilcock, Croning and Hing, 2006; Pournot et al., 2010). Bove, (2002) reported that water is 800 times denser than air, therefore exerting a greater force on the body. Additionally, water temperature alters the density, subsequently impacting the pressure acting on the body. Colder water is more dense than warmer water, water at 10°C has a density of 999.77 $kg.m^3$ whereas water of 35°C has a density of 994.08

kg.m^3 (McHutchinson, Martin and Barnwell, 1993). Therefore, the temperature of an immersion protocol will alter the level of hydrostatic pressure. The effects of hydrostatic pressure on the body are used to limit the formation of oedema, thus assisting the attenuation damage (Torres-ronda and Alcazar, 2014). Furthermore, hydrostatic pressure stimulates the displacement of fluids from the peripheral to the central cavity (Wilcock, Cronin and Hing, 2006). The shift of fluid from the peripheral to the central cavity increases the removal of the bi-products of the metabolism (Kaczmerek, Mucha and Jarawka, 2013,) whilst additionally increasing the transport of oxygen, thus aiding recovery. Moreover, hydrostatic pressure may assist in the re-absorption of interstitial fluid, further aiding the reduction in the formation of oedema (Frieden and Lieber, 2001). The compressive force exerted, increases the pressure gradient between the interstitial compartments in the legs and the intravascular space (Wilcock, 2006), whereby oedema is reduced.

It is acknowledged that water immersion simulates a feeling of weightlessness (Frangolias and Rhodes, 1996), as a result of Archimedes principle. This principle states that the upward thrust that a solution exerts on the body, is equal to the weight of the solution displaced (Wilcock, Cronin and Hing, 2006). Research has reported that this feeling of weightlessness stimulates greater relaxation and subsequently a reduction in perceptions of fatigue (Elias et al., 2012) as observed within the literature. A reduction in perceptions of fatigue and pain were present after participants performed; 10 min at 30°C or 24 min in warm water immersion, respectively (Nakamura et al., 1996; Kuligowski et al., 1998). These attenuations may be a result of a reduction in the NM activation that occurs when participating in water immersion (Wilcock, Cronin and Hing, 2006).

The magnitude of the force applied on the body is related to the depth of the water and the density of the solution in which an individual is immersed. This is calculated using the

following equation, where P_{hyd} is hydrostatic pressure, P_{atm} is atmospheric pressure (~1,013 Pa at sea level), g is gravity (9.81 m/s^2), p is water density (dependent on temperature) and h is height or depth of water.

$$P_{hyd} = P_{atm} + g \bullet p \bullet h$$

(Versey, Halson and Dawson, 2013)

The equation identifies water depth and density as factors that alter hydrostatic pressure. For every 1 cm of depth hydrostatic pressure increases by 0.74 mmHg (Wilcock, Cronin and Hing, 2006; Versey, Halson and Dawson, 2013). Furthermore, the literature has outlined positive effects of standing immersion protocols on recovery from EIMD when compared to passive recovery (Leeder et al., 2015). Vaile et al. (2008b) reported a significant positive effect of standing (shoulder level) CWI (table 1) and CWT protocols (15˚C, 1 min: 38˚C, 1 min; total 14 min) on recovery from DOMS. Although, research has identified a positive effect of a standing immersion protocol over passive recovery (Vaile et al., 2008b), research has failed to outline significant changes in hydrostatic pressure between seated immersion and standing immersion (CWI seated and standing; 14˚C for 8 min) protocols (Leeder et al., 2015). Leeder et al. (2015) reported that the hydrostatic pressure at the ankle was approximately 40 mmHg in the seated protocol; the hydrostatic pressure during the standing protocol at the ankle was reported to be approximately 111 mmHg. Despite a difference in pressure, no significant differences between groups were reported, therefore suggesting that the magnitude of the pressure changes need to be greater in order to stimulate a physiological response. Therefore, further methods of manipulating and assessing hydrostatic pressure should be explored. Recent research by Kane et al. (2016) has aimed to isolate the effect of hydrostatic pressure and temperature by comparing cold air therapy (CAT) and CWI. Although, this goes some way to isolate the effects of temperature and hydrostatic pressure, it

does not account for mechanical changes in hydrostatic pressure within an immersion protocol.

To the best of the authors knowledge, no research has been conducted into the effectiveness of a TWI saline solution protocol (TSI), to increase the hydrostatic effects. Adding salt (sodium chloride, NaCl) to water increases the number of molecules in the solute, thus increasing the density of the solution (Torres-Ronda and Alcazar, 2014), exerting greater compressive effects on the body (Torres-ronda and Alcazar, 2014), and an amplified effect of perceived weightlessness. Immersion in a 30% saline solution, somewhat similar to the solution of the Dead Sea (Moses et al., 2006), has stimulated anecdotal reports of a sensation of floating and complete weightlessness, highlighting the exacerbated effects of perceived weightlessness in a saline solution. However, this assertion is lacking in scientific evidence. The higher the concentration of the solution, further increases density and therefore pressure. However, the maximum solubility of salt in water varies dependent upon the temperature of the water (Martinez, 2015) and is therefore necessary to consider when designing a saline immersion protocol.

2.3.4 Summary of Water Immersion

The body of literature regarding water immersion as a recovery intervention from EIMD is vast. Many protocols have been employed in an attempt to attenuate the symptoms of damage, with some good attempts to draw recommendations from the literature. However, as a result of this variation, more recently it has become apparent that there is growing conflict between the conclusions and subsequently the recommended immersion protocols to employ post EIMD. Little research draws conclusive evidence to suggest the main action of immersion protocols to stimulate recovery. Therefore, this study will aim to isolate both

temperature and hydrostatic pressure to identify the effects on recovery of these variables on recovery.

2.4 Hypotheses

2.4.1 Experimental Hypotheses

It was hypothesised that following EIMD, CWI and TSI will significantly improve performance, blood, inflammatory and perceptual markers of recovery compared to CON and TWI groups.

Additionally, following EIMD the CWI group will show significantly improved recovery of performance, blood, inflammatory and perceptual markers compared to a TSI group.

2.4.2 Null Hypothesis

There will be no significant effect of CWI, TWI and TSI compared to a CON group on recovery from performance, blood, inflammatory and perceptual markers of EIMD.

CHAPTER THREE: METHODS

This section will begin with a brief overview of each visit to the University of Bedfordshire Sport Science Laboratories. After which, all experimental procedures will be described in detail in section 3.3.

3.1.1 Participants

The present study employed a randomised, between groups, design. Participants were recruited using a volunteer sampling method from a population of 18-40 year old, recreationally active, non-resistance trained males. Recreationally active males were defined as males participating in 3-6 hours of structured physical activity per week (White, Rhind and Wells, 2014) and were not regular users of water immersion recovery techniques.

Based a similar study design from Leeder et al. (2015) a G* power calculation was used to determine the necessary sample size to detect significance. Power (1 - β) was set at 0.80 and error probability level was set at $\alpha = 0.05$ and indicated that 28 participants were required to detect a significant effect based on a medium effect size (*f*) of 0.69 (Cohen, 1969). The present study excluded participants who: smoked, took non-steroidal anti-inflammatory drugs (NSAIDs), had previously used water immersion techniques or any supplement/compression garments that may assist in recovery from muscle damage, had partaken in EIMD studies 6 month prior to the present study. To ensure safety at all times throughout the experiment, participants were also excluded from the experiment if they suffered from: previous or existing lower limb/back injuries or cold intolerances such as chilblains or Raynauds syndrome.

Participants were asked to refrain from strenuous exercise 24 h prior to testing and refrain from strenuous exercise for the duration of the 2 week study, and were also asked to refrain from the consumption of caffeine 24 h prior to exercise and alcohol for 48 h prior to exercise. This study was granted ethical approval by the University of Bedfordshire Institute for Sport and Physical Activity Research (ISPAR) ethics committee. Participants were informed of the risks and asked to complete: informed consent, PAR-Q, blood screening and a risk assessment questionnaire.

Twenty five recreationally active males (Age: 22 ± 3 years; Mass: 79 ± 12 kg; Height: 1.8 ± 0.6 m) attended the University of Bedfordshire Sport and Exercise Science Laboratories on 6 occasions (figure 2). Visits 1 and 2 were familiarisation and pre-trials. Visit 3 was the main trials and intervention, and visits 4, 5 and 6 were 24, 48 and 72 hours post intervention analysis. Participants were randomly assigned to one of four groups (control (CON), cold water immersion (CWI), thermo-neutral water immersion (TWI) or thermo-neutral saline solution immersion (TSI) using an online random number generator.

3.2 Overview of Visits

3.2.1 Visit 1 and 2: Familiarisation and Pre-trials

Prior to participation, participants provided a urine sample and had their osmolality measured to ensure they were sufficiently hydrated. Blood pressure was also measured prior to exercise testing. Baseline measurements of height (m), mass (kg) and skin fold calliper measurements at the site of the thigh were taken. Secondly, a finger-tip capillary blood sample was obtained to analyse blood lactate (BLa) and creatine kinase (CK), followed by baseline measurements for limb circumference and cross sectional area (CSA) of the rectus femoris. Further to this, participants were required to perform baseline measures which included a $\dot{V}O_{2max}$ test, a test

of maximal power via a countermovement jump (CMJ) and a test of maximal strength via a maximal voluntary contraction (MVIC) of the quadriceps.

3.2.2 Visit 3: Main Trials and Intervention

Prior to commencing exercise participants had measurements of blood pressure and urine osmolality obtained. Participants performed a 5 minute self-paced warm up on a motorised treadmill (PPS55 Med-i Woodway, Cranlea), after which they performed 5 X 8 minutes (Willems and Northcote, 2013; Eston and Peters, 2000) of downhill running (-10% (Braun and Dutto, 2003) at 60% V_{max}) (HP Cosmos, Pulsar, HaB Direct).

Within 30 minutes of completing the downhill run, participants were either immersed for 15 minutes in: CWI (10-15˚C) (n = 6), TWI (35˚C) (n = 6) or TSI (35˚C, 30% saline solution) (n = 7). Participants in the control (CON) group were seated for 15 minutes (n = 6).

Throughout the immersion protocols participants had their heart rate (bpm) and core temperature (T_{core}) monitored via a self-inserted rectal probe 10 cm beyond the anal sphincter (Hanna, Checktemp- dip Hi- 98509-01, J-P Lennard), thermal sensation (appendix A) was also recorded throughout the immersion protocol.

3.2.3 Visit 4, 5 and 6: 24, 48 and 72 hours post

During visits 4, 5 and 6 to the Sports Science Laboratories, participants again had blood pressure and urine osmolality measurements, after which they were required to repeat the maximal strength assessments as performed in visits 1 and 2 and they were asked to report perceived soreness and perceived recovery 24, 48 and 72 hours post via a visual analogue scale VAS (appendices B and C).

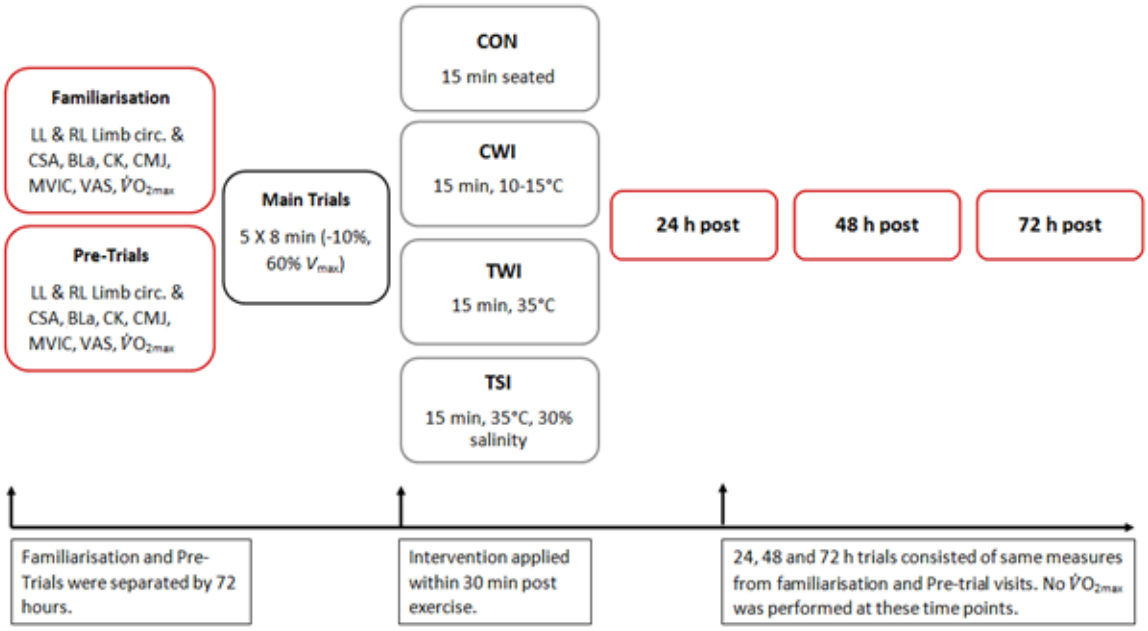


Figure 2. A schematic to demonstrate an overview of the study.

Note: LL; left leg, RL; right leg, CSA; cross sectional area, BLa; blood lactate, CK; creatine kinase, CMK; countermovement jump, MVIC; maximal voluntary isometric contraction, VAS; visual analogue scale, V_{max}; velocity max, CON; control, CWI; cold water immersion. TWI; thermo-neutral water immersion, TSI; thermo-neutral saline immersion.

3.3 Experimental Procedures

3.3.1 Blood Pressure and Urine Osmolality

The Omron M5-1 (Blood Pressure Monitors Auto, Omron, M5-I, Cranlea) was used to measure blood pressure in the brachial artery. The Omron M5-1 has been approved as a valid automated sphygomometer (El assaad, Topouchian and Asmar, 2003). Blood pressure was measured following the protocol outlined by the European Society of Hypertension (ESH) for use of automated devices with inflatable cuff for the upper arm (Parati et al., 2008). The participant was seated, and rested for at least 5 minutes before the measurement was taken with back supported, cuff at heart level and legs uncrossed. During the measurement the arm was supported on a table to ensure no isometric contractions of the muscles of the shoulder

and upper arm were performed (Parati et al., 2008). If a participants blood pressure reading was >140/90 mmHg, following a further 5 minutes of rest, the measurement was taken again. If the reading was still above the stated criteria to exercise, they were excluded from participation in the study (ACSM).

Urine osmolality was measured using a urine refractometer (Atago Vitech scientific, Pocket PAL-OSMO, HaB Direct) to establish whether participants were of the appropriate hydration status to exercise. Participants were required to have a urine osmolality reading of < 600 $mOsm.kgH_2O^{-1}$ to be deemed euhydrated in accordance with the University of Bedfordshire Sport Science Laboratories ethical guidelines. If participants were not deemed to be sufficiently hydrated they were asked to drink 500 ml of water and the test was repeated 20 minutes later, if the reading remained above 600 $mOsm.kgH_2O^{-1}$ they were not be able to commence exercise testing on that occasion.

3.3.2 Stature and Mass

Upon arrival at the laboratories participants had their stature measured using a wall mounted stadiometer (Harpenden, HAR-98.602, Holtain). For both height and mass, participants were instructed to remove footwear. Participants were required to stand in the anatomical position with a straight back and keeping heels in contact with the floor. Body Mass was measured using electronic, digital scales (Tanita, BWBO800, Allied Weighing).

3.3.3 Skin Fold Caliper Measurement

Skin fold caliper measurements (skin fold caliper, Harpenden, Cranlea), were taken at the site of the anterior aspect of the thigh only. This was necessary in order to establish body composition at the site of the muscle damage. The literature suggests that the quantity of adipose tissue surrounding the area of damage could significantly impact the rate of intra-

muscular cooling (Myrer, Myrer and Meason, 2001), therefore was a necessary measure to understand the impact of body composition on the effectiveness of the recovery (Leeder et al., 2011).

The site of measurement was located and marked with a cross, with two lines intersecting at right angles measured from the midpoint of distance between the iliac crest and the posterior border of the patella. The measurement was recorded in a seated position with the knee flexed to 90˚. The participants' foot was placed onto a support, after which they were asked to clasp their hands under their hamstrings and gently lift in order for the measurement to be taken. Measurements were taken on the right side of the body. Skin fold measurements were taken in accordance with the BASES guidelines (Winter et al., 2007), the measure was taken 3 times and an average of the 3 measures was reported.

Fifteen participants (21 ± 2 years; 76 ± 14 kg; 1.76 ± 0.11 m) were recruited to assess skin fold reliability via a volunteer sampling method. Participants attended the laboratory on three occasions for a baseline measure, 1 hour post and 3 hours post baseline measurement. Test re-test reliability of skin fold calliper measures was good with a CV of 2.0 % and an ICC of 0.99.

3.3.4 $\dot{V}O_{2max}$ Testing

$\dot{V}O_{2max}$ was measured as a preliminary test during the first visit, by using the analysis of expired air during a maximal incremental test to exhaustion (Cortex Metalyser Online Gas Analyser, Cortex, Metalyser 3B, Cranlea).

The $\dot{V}O_{2max}$ test consisted of a 5 minute self-paced warm-up. Initial work rate was established using a self-selected starting pace to ensure that test ran for approximately 10-12 minutes for each individual. Every minute the treadmill increased by 1 $km.h^{-1}$ until the participant reached volitional exhaustion (Midgely et al. 2009). The test was performed at a 1% gradient (Jones and Doust, 1996).

During the test RPE was measured using the Borg Scale (Borg, 1970) (appendix D) at each stage of the incremental test. Heart rate (HR) (Polar, FS1, Cranlea) was monitored continuously.

3.4 Outcome measures

3.4.1 Limb Circumference

Participants were asked to stand in the anatomical position for limb circumference to be measured at the mid-point between the iliac crest and the proximal border of the patella on the left and right leg (French et al., 2008).

Test re-test reliability data was conducted between baseline and pre-trials visits to determine repeatability of the measure. The CV of left leg limb circumference was 0.5%, while right leg limb circumference was calculated as 0.4 %.

3.4.2 Cross Sectional Area of Rectus Femoris

Cross sectional area of the rectus femoris was measured using B-mode ultrasonography (GE medical, Vivid 7 Echocardiograph system, Mius Ltd.) using a 10 Hz, 50 mm linear probe. The probe was placed 3/5ths of the distance from the illiac crest to the proximal border of the patella (Seymour et al., 2009). Participants were seated with their feet flat on the floor. Excess ultra-sound gel was applied to minimise tissue compression and image distortion (Seymour et al., 2009). Two, to three images were acquired to obtain each end of the rectus

femoris, scanning depth was set to ensure the femur was visible for orientation purposes. DICOM images were analysed offline using photoshop (Extended edition CS6 version 13.0.1, San Jose, CA). Images were stitched using the opacity tool, ensuring landmarks were aligned prior to manually tracing the border of the rectus femoris muscle to obtain CSA measurement (figure 2).

Test-retest reliability, of CSA analysis was analysed from fifteen participants (21 ± 2 years; 76 ± 14 kg; 1.76 ± 0.11 m). Participants were required to attend the laboratory on three occasions for a baseline measure, 1 hour post and 3 hours post baseline measurement. Test-retest reliability of CSA of rectus femoris via ultrasound scans was good with a CV of 3.7 % and an ICC of 0.99.

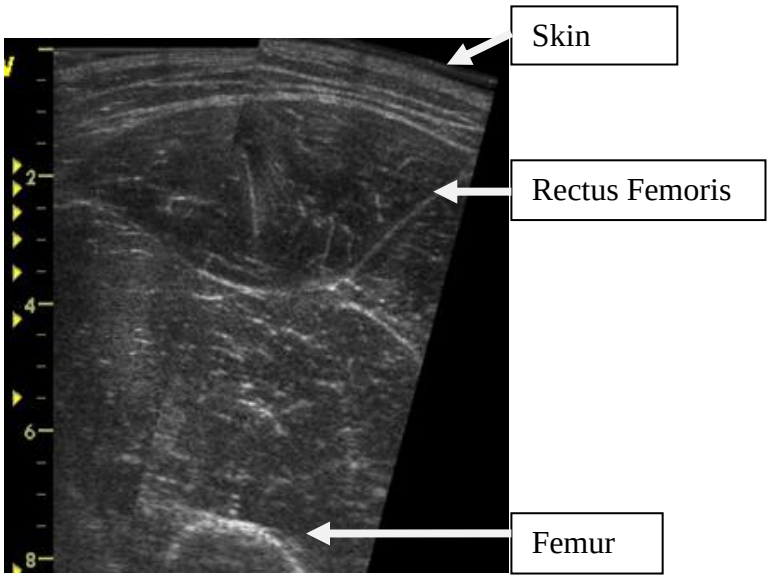


Figure 3. An example of images obtained through ultra sound scanning for analysis of cross sectional area.

Test re-test reliability data was also conducted between baseline and pre-trials visits. The CV of left leg CSA was 2.9%, while right leg CSA was calculated as 2.2 %.

3.4.3 Blood Collection

Each participant's hand was warmed in a bowl of warm water to increase blood flow. Participants finger-tip was cleaned using an alcohol wipe; this was then allowed to dry before a single use, disposable lancet was used to make a capillary puncture. Two microcuvettes were used to collect 40 µL of blood in each. A pipette was used to extract the sample from the capillary tube onto a test strip (Eston and Peters, 1999) to analyse CK (Relflotron, Reflotron Plus, Roche).

Test re-test reliability data was conducted between baseline and pre-trials visits to determine repeatability of the measure. The CV of CK between baseline and pre-trials was calculated as 40.3%.

Blood lactate analysis was conducted using the YSI (YSI, 2300 stat plus, YSI).

Test re-test reliability data was conducted between baseline and pre-trials visits to determine repeatability of the measure. The CV of CK between baseline and pre-trials was calculated as 31.1%.

3.4.4 Maximal Voluntary Isometric Contraction

Maximal voluntary contraction (MVIC) of the quadriceps was assessed at the knee, at a joint angle of 110˚ of extension using an isokinetic dynamometer (Goodall and Howatson, 2008; Kane et al., 2016) (Chattecx Corporation, Kin com 125E plus, Chattanooga). The centre of the joint was marked to ensure consistency, participants seated in an upright position at 90° hip flexion on the isokinetic dynamometer. Participants performed 3 maximal contractions on each leg, for a duration of 5 seconds. Each contraction was separated by 1 minute rest (Howatson, Goodall and van Someren, 2009). The machine was set up to suit the individual anthropometric measures of each participant.

Test re-test reliability data was conducted between baseline and pre-trials visits to determine repeatability of the measure. The coefficient of variation (CV) of MVIC on the left leg was calculated as 12.6 %, while right leg reported a CV of 11.9 %.

3.4.5 Countermovement Jumps

Participants performed 3 countermovement jumps (CMJ) (Probiotics, Just Jump, Cranlea); the highest of the three jumps was recorded. Participants were required to place their hands on their hips during the protocol to prevent the influence of arm movement. CMJ were separated by one minute in order to give participants time to recover (Pournot et al., 2011).

Test re-test reliability data was conducted between baseline and pre-trials visits to determine repeatability of the measure. The CV of CMJ performance was calculated as 3.4 %.

3.4.6 Perceptions of Pain and Recovery

To measure delayed onset muscle soreness, participants were asked to record perceived soreness via a visual analogue scale (VAS) on a scale of 0= no pain to 200 mm= worst possible pain, whilst performing a squat to 90° knee flexion (Leeder et al., 2015).

Perceived recovery was also be reported on a VAS scale of 0 mm= not at all recovered to 200 mm= fully recovered.

3.5 Interventions

3.5.1 Muscle Damaging Exercise Protocol- Downhill Running

Participants were required to perform 5 X 8 minute downhill running (HP Cosmos, Pulsar, HaB Direct) at a gradient of -10%, separated by 2 minutes of rest (Braun and Dutto, 2009), at 60% of the participants treadmill velocity max (V_{max}) established in the $\dot{V}O_{2max}$ test in visits 1 and 2 in accordance with research by Willems and Northcote (2013), to induce sufficient

levels of muscle damage. A 5 minute self-paced warm up was conducted at a 1% gradient (Jones and Doust, 1996).

3.5.2 Water Immersion

After completing the downhill run, participants changed into swim wear. Participants had core temperature monitored throughout the immersion protocol using (Hanna, Checktemp-dip Hi- 98509-01, J-P Lennard). The intervention was stopped if participants core temperature increased by more than 2˚C from baseline value or increased above 37.9˚C or if core temperature decreased by more than 1.5˚C from baseline values or below 35˚C in accordance with the University of Bedfordshire's ethical guidelines.

Participants in the CWI group were immersed in water of 10-15°C (13.0 ˚C ± 1.7 ˚C) in line with recommendations of Versey, Halson and Dawson, (2013) and Kaczmereck, Mucha and Jarawka (2013) who demonstrated that 15 minute immersion in water of 15°C was enough to reduce intramuscular temperature by approximately 10°C.

TWI is considered within the literature to be immersion in water approximately 35°C (Corbett et al., 2012; Versey, Halson and Dawson, 2013). In the present study both TWI and TSI groups were immersed in water of 35°C for 15 minutes. In this experiment 0.3 kg of sodium chloride per 1 L of H_2O to utilise a 30 % saline immersion protocol as there is evidence to state that immersion in a 30% solution is safe for participants (Matz, Orion and Wolf, 2003; Gamblicher, Terras and Skrygan, 2013). The control group were seated for 15 minutes post downhill run with no water immersion.

Immersion protocols took place in a 360 L water butt, with participants immersed to 1.20 m depth. The height of the water was marked on each participant and individual limb length was reported from maximum water height, to the mid-point of the thigh, established in prior measures of limb circumference, to calculate the differences in hydrostatic pressure at the site of the damage. The following equation, reported by Versey, Halson and Dawson (2013), was used to calculate hydrostatic pressure:

$$P_{hyd} = P_{atm} + g \times \rho \times h$$

Where P_{atm} refers to atmospheric pressure (~ 1013 Pa at sea level), g = gravity (9.81), ρ = density of the liquid (appendix F) and h = height of the liquid

3.6 Statistical Analysis

Reliability of skin fold calliper and ultra sound scan measures, test-retest coefficients of variation and intraclass correlation (Hopkins, 2000) were calculated.

IBM SPSS statistics version 21 (Chicago, IL) was used to analyse the physiological responses stimulated in each immersion protocol. Participant's descriptive statistics were presented mean standard deviation (M ± SD).

Normality of data was confirmed using Q-Q plots. A two-way repeated measures analysis of variance (ANOVA) (4 x recovery group, 4 x time), using recovery group as between subjects factor and time as the within subjects factor was utilised to compare the main effects of time, group and an interaction effect (time x group). Statistical significance was accepted at $p \leq 0.05$. Sidak's post hoc test was used where a statistically significant main effect was present.

To determine the practical implications, confidence limits (CL) were reported at 95%. Partial eta^2 effect sizes (η_p^2) were reported, effects of 0.2 were considered small effect, 0.5 a medium effect and > 0.8 considered a large effect (Durlack, 2009). In cases where Mauchley's test of sphericity was violated, or not reported, Greenhouse-Geisser correction was applied (Field, 2012).

Each participants score was presented as a percentage of baseline values. Baseline values were considered 100% in order to reduce the effect of group bias, with the exception of measures of perceptions of pain. Worst possible pain was reported as 100 % and no pain was reported as 0 %. Analysis of recovery is based on perception of recovery at 24, 48 and 72 h post compared to pre-damage state of 100% recovered.

CHAPTER FOUR: RESULTS

Table 2. Participant characteristics for CON (n= 6), CWI (n = 6), TWI (n = 6), TSI (n = 7) groups (M ± SD)

Group	Hydrostatic pressure (mmHg)	Height (m)	Mass (kg)	Age (years)	$VO_{2\ max}$ ($mL.kg.min^{-1}$)	Skin Fold (mm)	V_{max} ($km.h^{-1}$)
CON	0 ± 0	1.78 ± 0.6	83.87 ± 12.37	22 ± 2	45 ± 6	14 ± 6	15.3 ± 2.2
CWI	90 ± 3	1.82 ± 0.6	84.25 ± 12.84	22 ± 2	49 ± 5	13 ± 6	16.5 ± 1.8
TWI	89 ± 2	1.80 ± 0.5	78.71 ± 13.00	23 ± 2	50 ± 6	12 ± 3	16.8 ± 1.2
TSI	113 ± 4	1.79 ± 0.9	78.4 ± 11.75	25 ± 5	48 ± 5	11 ± 3	16.4 ± 1.5

Note: CON = control group, CWI = cold water immersion, TWI = thermo-neutral water immersion, TSI = thermo-neutral water immersion, V_{max} = Treadmill velocity max.

4.1 Markers of Swelling:

4.1.1 Left Leg Limb Circumference

No significant interaction effect of group over time was present for LL limb circumference ($F_{5.73,\ 40.11} = 0.709$, $p = 0.698$, $\eta_p^2 = 0.09$). Additionally, no significant main effect of group was present ($F_{3,\ 21} = 1.25$, $p = 0.32$, $\eta_p^2 = 0.15$). However, a significant main effect of time ($F_{1.91,\ 40.11} = 16.01$, $p < 0.01$, $\eta_p^2 = 0.43$) was reported (figure 3). The CON group demonstrated a significant increase in LL limb circumference between pre-trials and 24 hours post ($p = 0.03$, 95 % CL = -2.20 to -0.08). Additionally a significant increase from pre-trials to 48 hours post ($p = 0.002$, 95 % CL = -1.91 to -0.35) was present. However this significant increase in swelling had subsided by 72 hours post with a significant reduction in LL limb circumference occurring between 48 h post and 72 h post ($p = 0.02$, 95 % CL = 0.14 to 1.81). Immersion groups demonstrated no significant increase of LL limb circumference over time ($p > 0.05$).

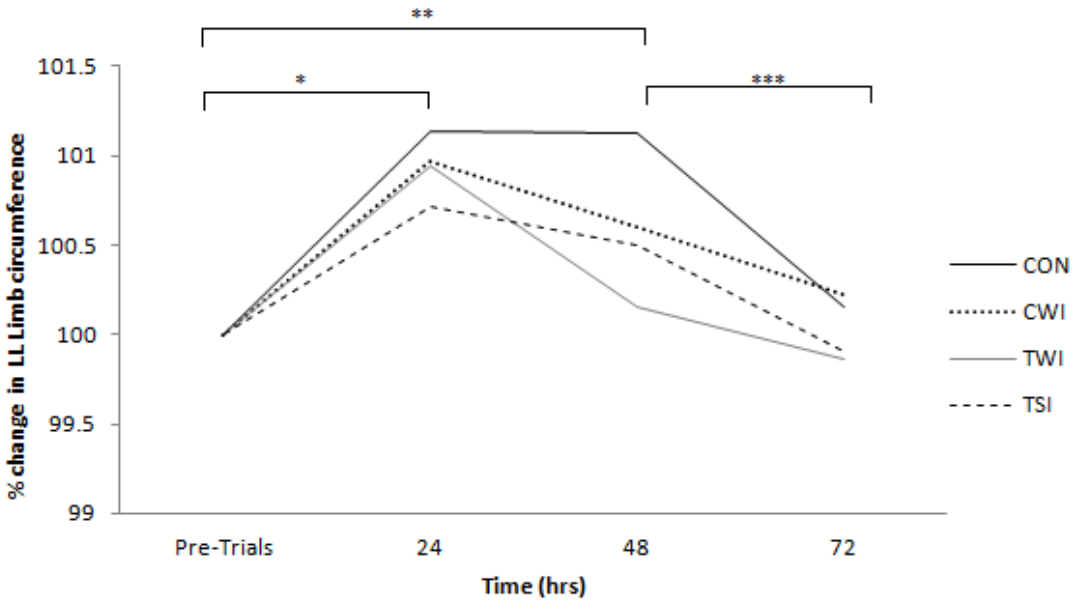


Figure 4. Percentage change in left leg limb circumference from pre-trials to post muscle damaging exercise.

**Denotes significant main effect of time in CON group from pre-trials to 24 h post. **Denotes significant main effects of time in CON group from pre-trials to 48 h post. ***Denotes significant main effect of time in CON group from 48 h to 72 h post damage.*

4.1.2 Right Leg Limb Circumference

No significant interaction effect of group over time was present for RL limb circumference ($F_{7.18,\ 50.27}$ = 1.29 , p = 0.27, η_p^2 = 0.16). No significant main effect of group was present ($F_{3,\ 21}$ = 0.613, p = 0.61, η_p^2 = 0.08). However, a significant main effect of time (figure 4) was reported ($F_{3,\ 63}$ = 24.99, p < 0.01, η_p^2 = 0.54). A significant increase in RL limb circumference was present from pre-trials to 24 h post across CON (p = 0.02, 95 % CL = -2.08 to -0.18), CWI (p = 0.02, 95 % CL = -2.07 to -0.11), TWI (p = 0.02, 95 % CL = -2.09 to -0.12) and TSI (p = 0.04, 95 % CL = -1.84 to -0.02) groups. Between 24 h and 72 h post RL limb circumference of participants in CWI (p = 0.02, 95 % CL = 0.14 to 2.08) and TWI

groups (p = 0.001, 95% CL = 0.53 to 2.47) had significantly reduced back to baseline measures.

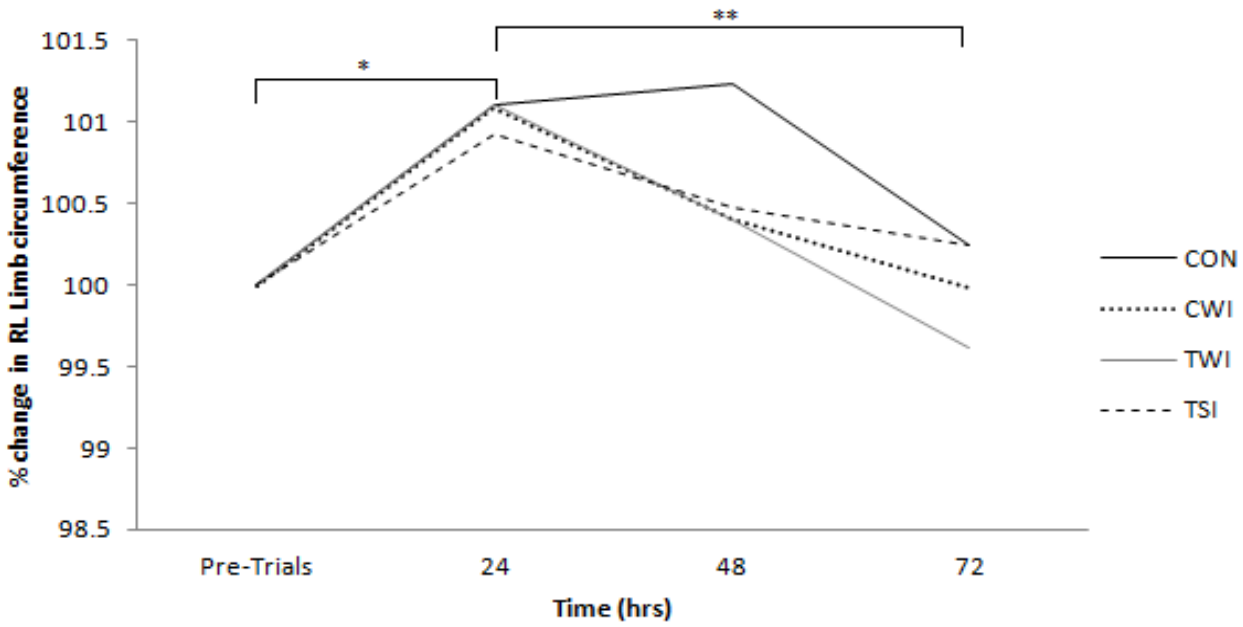


Figure 5. Percentage change in right leg limb circumference from pre-trials to post muscle damaging exercise.

**Denotes significant main effect of time from pre-trials to 24 h post in CON, CWI, TWI and TSI groups **Denotes significant main effect of time from 24 to 72 h post in CWI and TWI groups.*

4.1.3 Cross Sectional Area of Rectus Femoris in Left Leg

No significant interaction effect of group over time was present for analysis of CSA of the LL rectus femoris ($F_{5.57, 35.30}$ = 0.45, p = 0.83, η_p^2 = 0.07). No significant main effect of group was present ($F_{3, 19}$ = 0.04, p = 0.99, η_p^2 = 0.01). However, a significant main effect of time was present ($F_{1.86, 35.30}$= 17.69, p < 0.001, η_p^2 = 0.48) from pre-trials to 24 hours post (p < 0.001, 95 % CL = -8.52 to -2.80). Post hoc analysis revealed a significant increase in LL

CSA between pre-trials and 24 h post in CON (p = 0.02, 95 % CL = -11.72 to -0.75), CWI (p = 0.03, 95% CL = -11.58 to -0.60) and TSI (p = 0.02, 95% CL = -10.77 to -0.61) groups.

4.1.4 Cross Sectional Area of Rectus Femoris in Right Leg

No significant interaction effect of group over time was present for analysis of CSA of the RL rectus femoris ($F_{7.45,\ 47.18}$ = 0.97, p = 0.47, η_p^2 = 0.13). No significant main effect of group was present ($F_{3,\ 19}$ = 1.49, p = 0.25, η_p^2 = 0.19). However, a significant main effect of time was present ($F_{3,\ 57}$ = 27.04, p < 0.001, η_p^2 = 0.59). A significant increase in RL CSA was present in CON (p < 0.001, 95 % CL = - 9.33 to 3.60), CWI (p = 0.002, 95 % CL = -7.14 to -1.41), TWI (p = 0.009, 95 % CL = -7.96 to -0.95) and TSI (p < 0.001, 95% CL = -7.30 to -1.99) groups from pre-trials to 24 hours post. CON (p < 0.001, 95 % CL = -8.38 to -2.25), TWI (p = 0.001, 95 % CL = -9.62 to -2.11) and TSI (p < 0.001, 95 % CL = -7.97 to - 2.29) groups also reported further increases from pre-trials to 48 h post.

4.2 Markers of Performance

4.2.1 Countermovement Jump

No significant interaction effect of group over time was present in measures of CMJ performance ($F_{8.72,\ 61.00}$ = 0.81, p = 0.61, η_p^2= 0.10). No significant main effect of group was present ($F_{3,\ 21}$ = 1.82, p = 0.17, η_p^2 = 0.21). However, a significant main effect of time (figure 5) was present ($F_{3,\ 63}$ = 16.24, p < 0.001, η_p^2 = 0.44). CON group demonstrated a significant reduction in CMJ performance from pre-trials to 24 h post (p < 0.001 95 % CL = 4.58 to 15.32) . A significant reduction in CMJ performance was also present from pre-trials to 24 h post in TWI (p = 0.006, 95 % CL = 1.70 to 12.44) and TSI (p = 0.004, 95 % CL = 1.87 to 11.82). However this significant reduction had subsided in the TSI group from 24 h to

72 h post (p = 0.045, 95 % CL = -11.26 to -0.10). No main effect of time was present within the CWI group across time points (p > 0.05).

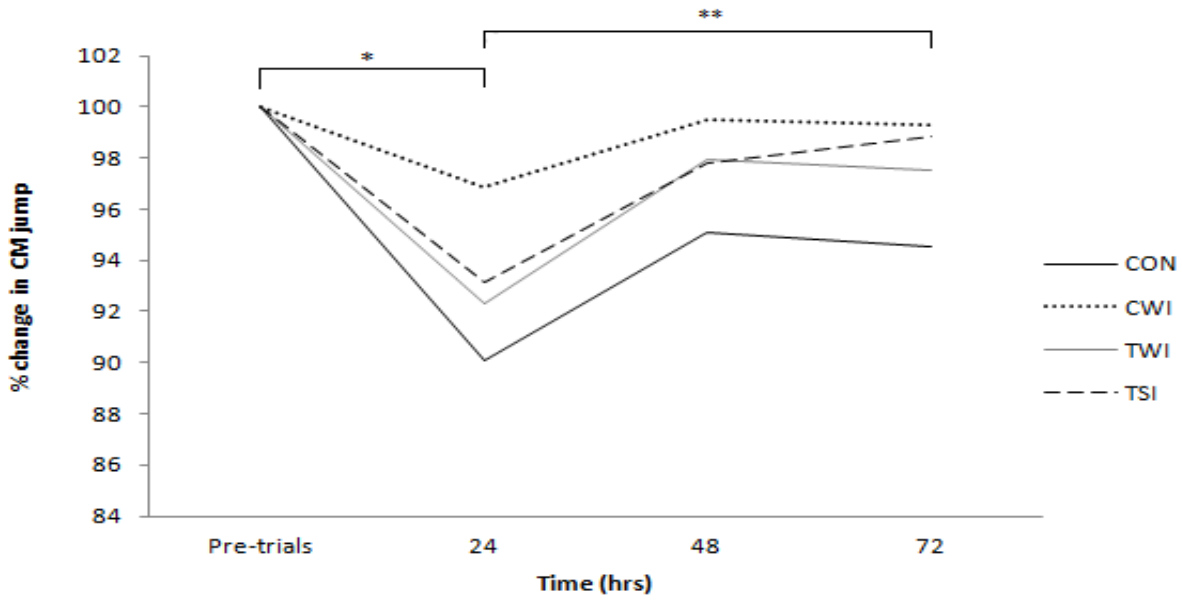


Figure 6. CMJ performance in CON, CWI, TSI and TWI groups post muscle damaging exercise.

** Denotes significant main effect of time in CON, TWI and TSI from pre-trials to 24 h post.*

*** Denotes significant main effect of time in TSI group from 24 h to 27 h post.*

4.2.2 MVIC Left Leg

No significant interaction effect of group over time was present in measures of left leg MVIC ($F_{8.49, 59.43}$ = 1.27, p = 0.28, η_p^2 = 0.15). Additionally, no significant main effect of group was present ($F_{3, 21}$ = 0.44, p = 0.73, η_p^2 = 0.06). However, a significant effect of time ($F_{3, 63}$ = 4.61, p = 0.006, η_p^2 = 0.18) was present across all groups resulting in a significant increase in

MVIC of LL from 24 h and 72 h (p = 0.01; 95 % CL = -14.85 to -1.60) post muscle damaging exercise. No further significance was reported ($p > 0.05$).

4.2.3 MVIC Right Leg

No significant interaction effect of group over time was present in measures of right leg MVIC ($F_{6.83,\ 47.78}$ = 0.88, p = 0.53, η_p^2 = 0.11). No significant main effect of group was present ($F_{3,\ 21}$ = 0.08, p = 0.97, η_p^2 = 0.01). Additionally, no significant main effect of time was present ($F_{3,\ 63}$ = 0.90, p = 0.45, η_p^2 = 0.04).

4.3 Blood Markers

4.3.1 Creatine Kinase

A significant interaction effect of group over time was present in measures of CK ($F_{7.06,\ 49.40}$ = 2.18, p = 0.05, η_p^2 = 0.24). A significant main effect of time (figure 6) was present (figure 6) ($F_{3,\ 63}$ = 22.61, $p < 0.001$, η_p^2 = 0.52). Participants in the CON group experienced a significant increase in CK level from pre-trials to: 24 h post (p = 0.001; 95 % CL = -198.01 to -4.66), 48 h post (p = 0.002, 95 % CL = -201.93 to -36.74), and 72 h post (p = 0.02, 95 % CL = -161.62 to -10.04). A significant effect of time was also present in the TSI group from pre-trials to 24 h post (p <0.001, 95 % CL = -201.27 to -59.30). However, this increase at 24 h had significantly reduced by 48 h post damage (p = 0.001; 95 % CL = 25.79 to 119.92) and further again from 24 h to 72 h post ($p < 0.001$; 95 % CL = 44.32 to 163.97). However, no significant effect of group was present in measures of CK ($F_{3,\ 21}$ = 2.40, p = 0.10, η_p^2 = 0.26).

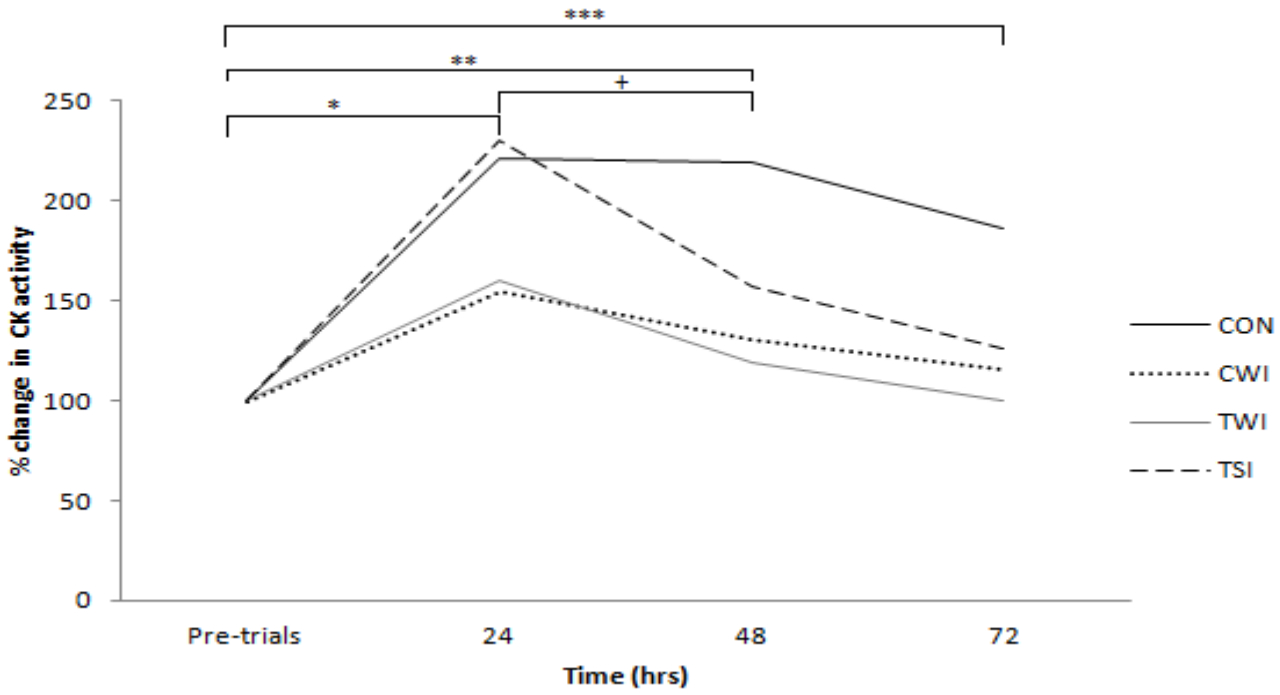


Figure 7. Percentage change in CK activity from pre-trials to post muscle damaging exercise. **Denotes significant main effect of time from pre-trials to 24 h in CON and TSI groups. ** Denotes significant main effect of time from Pre-trials to 48 h post in CON group. ***Denotes significant main effect of time from pre-trials to 72 h post. + Denotes significant reduction in CK activity from 24 to 48 h post in TSI group.*

4.3.2 Blood Lactate

No significant interaction effect of group over time was present in measures of blood lactate ($F_{5.69, 39.83}$ = 0.51, p = 0.79, η_p^2 = 0.07). No significant main effect of group was present ($F_{3, 21}$ = 0.10, p = 0.96, η_p^2 = 0.01). Additionally, no significant main effect of time was present ($F_{1.90, 39.83}$ = 2.28, p = 0.12, η_p^2 = 0.10).

4.4 Visual Analogue Scales

4.4.1 Perceptions of Pain

A significant interaction effect of group x time was present in perceptions of pain ($F_{6.11, 42.74}$ = 3.12, p = 0.01, η_p^2 = 0.31). A significant main effect of time ($F_{2.04, 42.74}$ = 61.54, p < 0.001,

η_p^2 = 0.75) was present in CON, TWI and TSI groups from pre-trials to 24 h post (p < 0.001, 95 % CL = -58.05 to -24.62; p < 0.001, 95 % CL = -61.38 to 27.95; p < 0.001, 95 % CL = -46.97 to -16.03) respectively. Additionally, CON and TWI groups reported a significant increase in perception of pain from pre-trials to 48 h post respectively (p = 0.02, 95 % CL = -45.78 to -2.72; p < 0.001, 95 % CL = -64.95 to -21.88). For participants in CON and TSI groups, pain at 24 h had significantly reduced by 48 h post (p = 0.006, 95 % CL = 4.17 to 29.99; p = 0.05, 95 % CL = 0.05 to 23.95) respectively. Perceptions of pain in the TWI group had significantly reduced from 24 h to 72 h post (p < 0.001, 95 % CL = 12.16 to 43.67), with the further significant reductions in pain occurring from 48 h to 72 h post (p < 0.001, 95 % CL = 12.20 to 41.13).

No significant increase (p = 0.08, 95 % CL = -32.05 to 1.38) in perceptions of pain in the CWI group from pre-trials to 24 h post were present (p > 0.05). Additionally, no significant effect of group was reported ($F_{3, 21}$ = 2.49, p = 0.089, η_p^2 = 0.26).

Table 3. Perceptions of pain post muscle damaging exercise (%)

	CON	CWI	TWI	TSI
Pre	6.7 ± 3.8	7.7 ± 7.0	2.4 ± 1.3	2.8 ± 3.0
24 h	48.0 ± 12.2	23.0 ± 17.8	47.1 ± 16.9	34.3 ± 15.8
48 h	30.9 ± 10.1	18.0 ± 18.8	45.8 ± 22.6	22.3 ± 21.7
72 h	17.3 ± 10.5	4.8 ± 4.8	19.2 ± 14.1	11.6 ± 12.9

Note: CON = control group, CWI = cold water immersion, TWI = thermo-neutral water immersion, TSI = thermo-neutral water immersion. Pre= Pre muscle damaging exercise, 24 h= 24 hours post muscle damaging exercise, 48 h= 48 hours post muscle damaging exercise, 72 h = 72 hours post muscle damaging exercise, data presented as % of pain (100 % 'worst possible pain' 0 % 'no pain') mean ± standard deviation

4.4.2 Perceptions of Recovery

A significant interaction effect of group over time was present in perceptions of recovery (figure 7) ($F_{5.82\ ,\ 40.76}$ = 3.02, p = 0.02, η_p^2 = 0.30) at 24 h post exercise between CON and

CWI groups (p = 0.02, 95 % CL = -83.87 to -6.63). This confirmed that CWI group reported significantly increased perceptions of recovery at 24 h post damaging exercise compared to a CON group. A significant main effect of group ($F_{3, 21}$ = 4.13, p = 0.02, η_p^2 = 0.37) was reported between CON and CWI (p = 0.02, 95 % CL = -41.07 to -3.09).

Additionally, a significant effect of time ($F_{1.94, 40.76}$ = 93.90, p < 0.001, η_p^2 = 0.82) was present across all groups from pre EIMD to 24 h post (p < 0.001, 95 % CL = 43.41 to 70.23), suggesting a significant effect of the muscle damaging protocol.

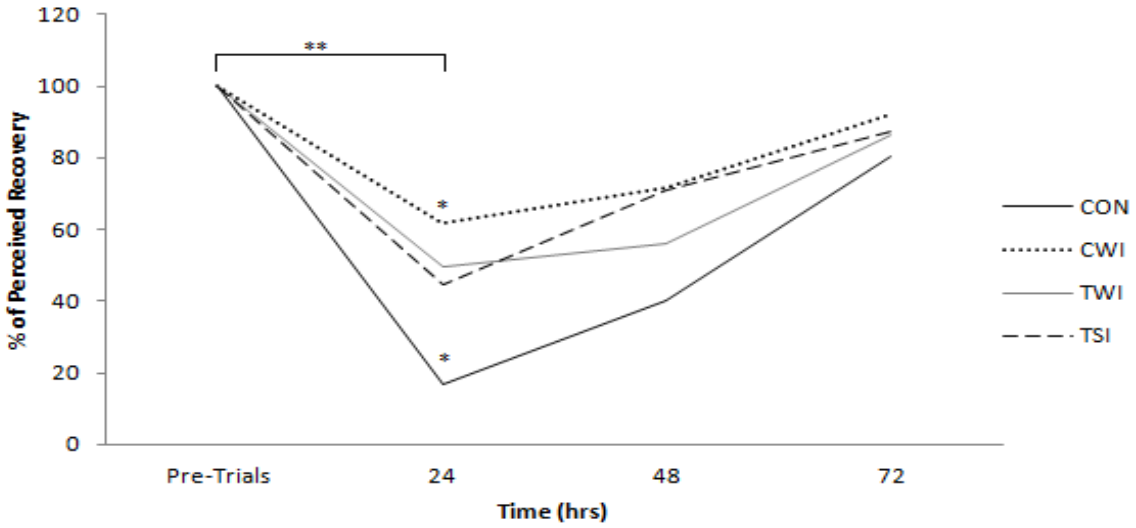


Figure 8. Percentage of perception of recovery in CON, CWI, TWI and TSI groups post muscle damaging exercise.

** Denotes significant main effect of group at 24 h between CON and CWI. ** Denotes significant main effect of time across all groups from Pre-trials to 24 h post.*

Table 4. Descriptive statistics (M ± SD) for all dependent variables measured post muscle damaging exercise

	LL. Limb Circ.	(cm)				**RL. Limb Circ.**	(cm)		
Time	CON	CWI	TWI	TSI	Time	CON	CWI	TWI	TSI
Pre	59.2 ± 6.7	60.6 ± 2.5	58.5 ± 5.0	59.7 ± 3.2	Pre	59.5 ± 6.2	61.7 ± 2.1	58.8 ± 5.0	60.1 ± 3.5
24 h	59.9 ± 6.7	61.2 ± 2.5	59.1 ± 4.9	60.1 ± 3.0	24 h	60.1 ± 6.1	62.3 ± 2.0	59.4 ± 4.8	60.6 ± 3.2
48 h	59.8 ± 6.6	61.0 ± 2.6	58.6 ± 5.0	50.0 ± 3.0	48 h	60.2 ± 6.0	62.0 ± 2.0	59.0 ± 5.0	60.3 ± 3.2
72 h	59.3 ± 6.8	60.8 ± 2.5	58.5 ± 5.3	59.6 ± 3.1	72 h	59.6 ± 6.3	61.7 ± 2.0	58.5 ± 4.9	60.2 ± 3.4
	LL. X- Sec. Area	(cm^2)				**RL. X-Sec. Area**	(cm^2)		
Time	CON	CWI	TWI	TSI	Time	CON	CWI	TWI	TSI
Pre	16.3 ± 1.7	15.9 ± 2.9	13.5 ± 3.8	15.2 ± 2.9	Pre	15.9 ± 2.6	16.9 ± 1.9	15.2 ± 3.6	15.7 ± 2.9
24 h	16.7 ± 1.4	16.6 ± 3.0	14.4 ± 3.8	16.1 ± 3.0	24 h	16.9 ± 3.1	17.6 ± 1.9	15.8 ± 4.1	16.5 ± 3.0
48 h	16.5 ± 1.8	16.4 ± 2.8	14.9 ± 3.9	16.2 ± 3.0	48 h	16.6 ± 2.8	17.3 ± 1.6	16.2 ± 3.6	16.5 ± 3.0
72 h	16.2 ± 1.8	15.9 ± 2.9	14.7 ± 3.7	16.1 ± 2.8	72 h	16.0 ± 2.4	17.0 ± 1.6	15.4 ± 4.2	16.2 ± 2.6
	CK	($IU.\ L^{-1}$)				**BLa**	($mmol.L^{-1}$)		
Time	CON	CWI	TWI	TSI	Time	CON	CWI	TWI	TSI
Pre	304 ± 207	311 ± 146	323 ± 127	275 ± 114	Pre	0.733 ± 0.265	0.693 ± 0.186	0.659 ± 0.202	0.607 ± 0.078
24 h	630 ± 355	419 ± 113	522 ± 235	618 ± 340	24 h	0.784 ± 0.226	0.804 ± 0.376	0.722 ± 0.288	0.660 ± 0.222
48 h	619 ± 389	346 ± 100	392 ± 191	423 ± 270	48 h	0.607 ± 0.077	0.600 ± 0.092	0.705 ± 0.233	0.582 ± 0.118
72 h	491 ± 252	325 ± 153	317 ± 162	366 ± 282	72 h	0.653 ± 0.111	0.569 ± 0.255	0.726 ± 0.340	0.573 ± 0.105
	RL MVIC	(N)				**LL MVIC**	(N)		
Time	CON	CWI	TWI	TSI	Time	CON	CWI	TWI	TSI
Pre	1177 ± 175	1405 ± 248	1417 ± 280	1486 ± 324	Pre	1021 ± 188	1248 ± 222	1243 ± 305	1361 ± 330
24 h	1077 ± 184	1451 ± 169	1371 ± 186	1397 ± 303	24 h	956 ± 142	1273 ± 198	1205 ± 224	1313 ± 326
48 h	1182 ± 182	1310 ± 106	1406 ± 179	1539 ± 253	48 h	1076 ± 1209	1208 ± 291	1315 ± 260	1447 ± 263
72 h	1188 ± 198	1431 ± 2200	1407 ± 240	1531 ± 263	72 h	1053 ± 266	1276 ± 257	1365 ± 228	1475 ± 255

	CM Jump	(cm)				**VAS Recovery**	(mm)		
Time	CON	CWI	TWI	TSI	Time	CON	CWI	TWI	TSI
Pre	44.3 ± 10.3	48.1 ± 5.4	52.1 ± 5.8	53.1 ± 10.6	Pre	200 ± 0	200 ± 0	200 ± 0	200 ± 0
24 h	40.0 ± 10.3	46.4 ± 4.2	48.3 ± 5.0	49.5 ± 9.9	24 h	33 ± 20	124 ± 53	99 ± 35	89 ± 61
48 h	42.0 ± 9.3	47.8 ± 5.9	51.1 ± 7.3	51.8 ± 10.0	48 h	80 ± 10	143 ± 63	112 ± 41	142 ± 31
72 h	42.0 ± 10.8	47.6 ± 4.6	50.7 ± 5.0	52.4 ± 10.5	72 h	161 ± 21	184 ± 16	173 ± 14	174 ± 24

Note: CON = control group, CWI = cold water immersion, TWI = thermo-neutral water immersion, TSI = thermo-neutral water immersion, LL= Left Leg, RL = Right Leg, CSA = Cross sectional area, CK = Creatine Kinase, BLa = Blood Lactate, CM Jump = Countermovement Jump, MVIC = Maximal voluntary isometric contraction, VAS = Visual analogue scale

CHAPTER FIVE: DISCUSSION

The main aim of the present study was to investigate the effect of water temperature and hydrostatic pressure on recovery from EIMD. Furthermore, the study aimed to investigate the effects of an increased hydrostatic pressure protocol (TSI) on recovery from EIMD. To the best of the authors knowledge, this was the first investigation of a saline immersion protocol post EIMD.

Overall, immersion groups demonstrated some attenuation of the markers of EIMD compared to the control group, however, this attenuation of damage was not deemed statistically significant. Therefore, the data cannot determine whether the effect of an immersion protocol on the markers of muscle damage is a result of the effects of hydrostatic pressure, or the effect of temperature. As a result, the null hypothesis was accepted. Nevertheless, despite this lack of statistical significance between groups, the main effects of time, present within variables, may suggest a greater beneficial effect of the water immersion protocols compared to passive recovery.

5.1 Blood Markers

Despite the attenuation of EIMD in immersion groups, analysis of CK presented an unexpected finding. The TSI group presented a significant increase in CK activity 24 h post damage, followed by a significant reduction from 24 to 48 h post. Without further research, little inference can be made regarding the mechanisms responsible for this impact of a TSI protocol on CK activity post damaging exercise. As the present study is the first to evaluate the use of a TSI protocol, therefore, there is no previous literature with which comparisons can be drawn. However, it could be speculated that a TSI protocol stimulates an increased CK response post EIMD, whereby an efflux occurs. As a TWI protocol did not demonstrate

the same trends in CK response, the efflux may be a result of the increased hydrostatic pressure, or more specifically the actions of salt. An increased hydrostatic pressure protocol, may have stimulated a greater redistribution and disruption of the blood. The increase in pressure may have magnified venous and lymphatic compression, increasing central blood volume, arterial pressure, cardiac volume and stroke volume (Becker and Cole, 1998). This enhanced response may account for the increase in circulating CK. However, the lack of direct evidence of the effects of a TSI protocol on the body it is difficult to infer explanation to account for the results presented.

Neither CWI or TWI groups exhibited such trends in CK activity. Nonetheless, across all groups the highest CK values were present at 24 h post, this finding is in line with that of the previous literature (Miyama and Nosake, 2004a; Bailey et al., 2007; Goodall and Howatson, 2008; Leeder et al., 2015). The results from the present research suggest that a CWI and TWI protocol can reduce the efflux of CK 24 h post exercise. The effect of CWI may be explained by the proposed mechanical actions of cold application that suggest vasoconstriction and a reduction in permeability of capillary walls contribute to a reduction in blood flow to the site of damage, thus attenuating CK activity (Eston and Peters 1999; Cochrane, 2004). This result is in line with that previously expressed within the literature (Vaile et al., 2008; Ascensao et al., 2011) that also concluded a positive effect of CWI on CK activity at 24 h post EIMD. Nevertheless, Bailey et al. (2007) did not report a significant attenuation of CK activity following a CWI protocol (10 min 10°C). The equivocal results of the literature presented may be indicative of individual variation in blood kinetics and CK response (Ascensao et al., 2011). This study aimed to account for high individual variation in resting CK activity by presenting data as percentage of baseline. However, this does not account for the variation in individual response post EIMD. It is possible that the results of CK are due to high variation

in individual response (Clarkson and Ebbling, 1988), opposed to the actions of immersion protocols, particularly in the TSI group. Additionally, issues surrounding the reliability of analysing CK using a Reflotron, highlighted in section 2.2.2, may account for variation in results. Therefore, CK response should be interpreted with caution.

The results of BLa analysis demonstrated no significant changes across time points between the groups, suggesting little impact of the muscle damaging protocol on measures of BLa at 24, 48 and 72 h post damage. However, Gleeson et al. (1998) reported a significant increase in BLa during a cycling exercise following, a bout of eccentric exercise compared to a control group of no eccentric exercise prior to cycling exercise ($p < 0.05$). It was suggested that this efflux was a result of increased muscle membrane permeability that occurred as a result of performing eccentric exercise (Newham et al., 1983). Although, measurements of BLa were obtained during cycling exercise, immediately post and 2 minutes post exercise. The present study did not report measures of BLa immediately post damage or immediately post immersion, this may account for the differences in results obtained between the studies. Future literature should address whether an efflux of BLa occurs immediately post damaging exercise, and immediately post intervention, particularly when considering recovery interventions for athletes required to perform multiple times within a day.

5.2 Measures of Performance

Based on the previous literature, it was expected that participants, particularly those in the CON group, would experience a significant reduction in MVIC at 24 and 48 h post damaging exercise (Plattner, Lambert and Baumeister, 2014; Leeder et al., 2015). In line with the literature, reductions at 24 h post were present in the CON (LL: -5.2 ± 14.3 %, RL: -7.4 ± 17.1 %), TWI (LL: -1.4 ± 9.2 %, RL: -1.2 ± 15.3 %) and TSI (LL: -1.8 ± 18.2 %, RL: -4.0 ± 18.7 %) groups, however, these reductions were not considered statistically significant. It

should also be considered that, again, although not significant, TWI and TSI would appear to report smaller deficits in performance compared to CON. However, the large SD in each group also suggests that there was vast variation in the performance of MVIC post EIMD. Meanwhile, CWI group demonstrated a mean improvement in performance of MVIC from pre-trials to 24 h post (LL: 2.8 ± 12.4 %, RL: 5.3 ± 17.4 %), although not statistically significant. This improvement in performance, does not reflect the results from previous research, however similar trends can be inferred from the literature that have demonstrated a positive effect of CWI on performance post EIMD (Vaile, Halson and Gill, 2008; Ingram et al., 2009; Brophey-Williams, Landers and Wallman, 2011). Nevertheless, some reports speculate that CWI may inhibit NM function and performance of MVIC immediately post intervention. This should be considered when utilising a CWI protocol for athletes performing at multiple times throughout a day (Wilcock, Cronin and Hing, 2006), although no observation of detrimental effect was reported in this investigation, in these circumstances, it may be more useful to utilise a TWI or TSI protocol.

Consistent with reports within the literature (Leeder et al., 2015; Byrne and Eston, 2002b), the results revealed an expected significant effect of time at 24 h post damage in the CON group (-10.0 ± 6.6 %) on performance of CMJ. CON group reductions at 24 h post damage are similar to those reported by Byrne and Eston, (2002b) of 10-15 %. Furthermore, TWI (-7.7 ± 3.7 %) and TSI (-6.8 ± 3.3 %) groups reported detrimental effects of EIMD on CMJ performance at 24 h post. Performance of CMJ in the CWI group also reported a deficit at 24 h (-3.2 ± 4.0 %) however, this deficit was not statistically significant. Nevertheless, future research should consider the observations of Byrne, Twist and Eston (2004) who observed that exercises that do not stimulate the stretch-shortening cycle (SSC), report greater detrimental effects compared to exercises such as CMJ, that do require the stimulation of the

SSC (Eston, Twist and Byrne, 2003) suggesting that the SSC may attenuate performance deficits. As the present study only reports one assessment of jump performance, the results can only account for changes in exercise performance that stimulates the SSC.

Overall, analysis of performance markers presented figures that may indicate that CWI is a more effective intervention than passive, TWI or TSI protocols, however, statistical analysis of between groups effects were not deemed to be statistically significant. This should be considered before recommending a recovery intervention.

When analysing the effect of EIMD on performance of MVIC and CMJ, despite lacking in statistical significance, the percentage change in performance may indicate to coaches and athletes that CWI could potentially result in the greatest attenuation of damage. This appears to suggest an early indication that temperature may have a greater effect over hydrostatic pressure on performance measures. Nevertheless, this consensus is not consistent across all of the literature. Crystal et al. (2013) reported no beneficial effect of a CWI protocol (20 min, 5°C) and concluded that immersion for 20 min at 5°C should not be recommended to attenuate the symptoms of damage. However, it is important to highlight the temperature of the protocol employed by Crystal et al. (2013). Immersion in 5°C is for 20 min does not fall into the recommendations proposed by Versey, Halson and Dawson (2013) of 5-15 minutes at 10-15°C, with the general conclusion that the lower the temperature the shorter the duration. Therefore, protocols utilising temperature's < 10°C to stimulate recovery should be carefully considered, due to the negative effects on contractile velocity (White and Wells, 2013) and potential effects on adaptation (Versey, Halson and Dawson, 2013)

5.3 Measures of Inflammation

Analysis of limb circumference and CSA of rectus femoris presented some unexpected outcomes. RL limb circumference reported a significant effect of time from pre-trials to 24 h

and from pre-trials to 48 h post across all groups. Additionally, analysis of CSA of RL rectus femoris demonstrated a significant effect of time from pre-trials to 24 h post across all groups suggesting that CWI, TWI and TSI protocols are ineffective in reducing swelling. This result was expected within the CON, TWI and TSI groups as there is little evidence to suggest the effectiveness of TWI, and no previous evidence of the effect of a TSI protocol on swelling. However, the significant increase in swelling was not hypothesised within the CWI group, as some literature has reported a beneficial effect of CWI on mid-thigh limb circumference at 24, 48 and 72 h post muscle damage (Vaile et al., 2008, Roberts et al., 2014). This conflict of outcomes may be due to a difference in immersion protocols and methodology employed. Vaile et al. (2008) subjected participants to a CWI protocol of a similar temperature and duration (15°C, 14 min) to the CWI protocol employed in the present study, however, Vaile et al. (2008) utilised full body (only head and neck out) immersion. The latter protocol achieved greater depth, and hydrostatic pressure on the body and subsequently, a greater displacement of fluid (Vaile et al., 2008). This may indicate an effect of hydrostatic pressure. Further, the present study and that of Vaile et al. (2008), measured thigh circumference at 3 sites (above the knee, mid thigh and sub gluteal), and did not report measures of CSA via ultra-sound scanning, the difference in experimental protocols employed may also account for the difference in outcomes. Analysis of CSA is a measure susceptible to experimenter error, analysis of 95% CL reported for CSA were large in terms of performance, therefore, the results may lack precision and should be interpreted with caution.

Analysis of RL limb circumference and CSA does reflect some of the reports within the literature suggesting that immersion protocols have little effect on measures of swelling (Howatson and van Someren, 2009; Crystal et al., 2013). If an intervention has little effect on swelling reports suggest that athletes may be susceptible to a reduction in ROM and

subsequently: increases in perceptions of pain, a deficit in exercise performance and maximal force production (Warren et al., 2001; Prisk and Huard, 2003). Therefore, the lack of attenuation of swelling could account for observed reductions in performance markers. Sufficient attenuation of swelling may reduce the occurrence of performance deficit, when employing recovery interventions this should be considered.

It was expected that a similar trend would be present in analysis of LL swelling, however, not all of the data were indicative of the same trends. A main effect of time in the CON was present from pre-trials to 24 h post and 48 h post, confirming the effect of muscle damaging exercise. However, CWI, TWI and TSI groups did not report any significant increase in LL limb circumference from pre-trials to post damage measures. This suggests a positive effect of immersion protocols on the attenuation of swelling. Nevertheless, this was not present in measures of LL CSA of rectus femoris. CON, CWI and TSI groups demonstrated a significant increase in LL CSA of rectus femoris from pre-trials to 24 h post. These data contradict the data obtained from analysis of LL limb circumference. The inconsistency between measures may be a result of issues of reliability surrounding the methodology employed in order to obtain measures of swelling. Although reliability measures were reported and appropriate actions were taken in order to obtain reliable measures, there are some instances where human error may account for these differences. In order to measure limb circumference and CSA at the same point on each visit to the laboratory, participants mid-point of each thigh was marked with permanent marker, however in some instances over the 2 week testing period, this point had to be re-marked, therefore the potential for error in measurement occurred. Nevertheless, CSA test-retest reliability prior to data collection reported a CV of 3.7%. Percentage change in CSA across groups was greater than this (table 4), therefore, it could be suggested that these changes were real changes.

The variation between LL and RL markers of swelling from pre-trials to post damage measures, although unexpected, may have potential explanations regarding dominance. The literature to date generally reports measurements of swelling from the non-dominant side (Howatson and van Someren, 2009; Crystal et al., 2013). This may suggest that damage has a greater effect on the non-dominant side, and may account for the differences between RL and LL. However, little explanations can be inferred using the results of the present study, as dominant leg was not reported. In order to account for this, future research should report dominance/significant differences in anthropometrics between sides, and report measures on both sides in order to establish the effects of dominance.

5.4 Measures of Pain

Analysis of pain scales identified significant increases in pain in CON, TWI and TSI groups from pre-trials to 24 h post. In line with previous literature (Ingram et al., 2009; Ascensao et al., 2011; Stanley et al., 2012) CWI group did not report a significant increase in pain post damaging exercise. This may be explained by the proposed analgesic effect of a CWI protocol (Cook and Beaven, 2013) due to a reduction in muscle contractile velocity and reduced muscle spasticity (Wilcock, Cronin and Hing, 2006). It was also expected that a TSI protocol would demonstrate greater attenuation of pain over TWI and CON groups due to the increased hydrostatic pressure that assists with the fluid shift from the peripheral to the central cavity, reducing accumulation of metabolites and perceptions of pain (Elias, 2013), however, these effects were not evident 24 h post. This may be a result of the efflux of CK that occurred 24 h post, which is linked with perceptions of pain, as at 48 h post the TSI group reported significant reductions in perceptions of pain in line with a significant reduction of CK 48 h post. Further explanations that may account for this reduction of pain in immersion groups may refer to the sensations of perceived weightlessness and reduced

perceptions of fatigue reported when performing water immersion protocols (Frangolias and Rhodes, 1996). Moreover, when immersed in a 30% saline solution such as the Dead Sea, reports of a floating sensation are prevalent (Moses et al., 2006). Therefore, it is reasonable to suggest that perceptions of pain are further reduced in a TSI protocol and report a quicker recovery time over a TWI or CON protocol. Overall, the results demonstrate enhanced perceptions of pain in all immersion groups over CON group.

5.5 Measures of Recovery

All groups reported a significant main effect of time from pre-trials to 24 hours post damage, demonstrating the effect of the damage protocol. A significant interaction effect was detected between CON and CWI groups at 24 h post exercise, suggesting that a CWI is significantly more effective at improving perceptions of recovery than performing no intervention, somewhat similar to the reports from previous literature (Stanley et al., 2012). Reports suggest that if an intervention improves perceptions of recovery, this may have a positive effect on subsequent performance (Cook and Beaven, 2013). Analysis of TWI and TSI groups, although lacking in statistical significance, demonstrate improved perceptions of recovery over a CON group. Furthermore, although at 24 h post CWI (61.8 ± 26.7 %) would appear to be the most effective immersion protocol to employ, at 48 h post, perceptions of recovery in the TSI group (70.8 ± 15.5 %) are similar to that of CWI group (71.7 ± 31.6 %). Additionally, while TWI group reported greater perceptions of recovery over the TSI group at 24 h post, the TSI group reported greater perceptions of recovery at 48 h post over the TWI group (56.1 ± 20.7 %). This should be considered when employing an intervention to maximise recovery of subsequent performance whilst additionally taking into account an athlete's comfort and preference of intervention. Finally, improved perceptions of recovery at 72 h were reported in CWI (91.9 ± 7.8 %), TWI (86.6 ± 6.8 %) and TSI (87.1 ± 12.0 %)

groups over CON group (80.5 ± 10.6 %), indicating a faster recovery in immersion groups. However, the lack of statistical significance between groups would suggest that this should be interpreted with caution. It is also important to consider the methodological issues with reporting perceptions of pain and recovery using VAS measurements. Reporting perceptual measures relies on individual interpretation, therefore data are subjective (Cleak and Eston, 1992) and should be considered alongside physiological measures of damage (Cook and Beaven, 2013). In order to minimise this effect, participants were shown identical scales on each visit, the scale was continuous opposed to a numbered scale, as continuous scales have been reported to provide a more reliable measure of pain over time (Zusman, 1986). In addition to the subjectivity of VAS as measure of damage, participants can also be influenced by subject expectancy effect. Camf et at. (1991) reported positive expectancy had a powerful effect on individual behaviour and beliefs, when investigating the effects of a placebo on smoking behaviour. When considering the results of Camf et al. (1991) it should be noted that the expectation that CWI can benefit subsequent performance, may contribute to subsequent positive or negative performance (Cook and Beaven, 2013). CWI protocols as recovery methods for athletes are widely reported on social media and within the news, such as the report of Andy Murray pictured in an ice bath with the Wimbledon trophy in June 2016 (appendix E), this may stimulate expectation that a CWI protocol is beneficial to performance.

It is possible that participants in the CWI group had a greater belief that a CWI protocol could benefit them over less familiar TWI and not at all reported TSI protocols, worthy of further study. Therefore, participants of the present study may have been affected by a cognitive bias referred to as "prior hypothesis and focusing on limited targets" (Das and Teng, 1999, pp. 762). This refers to the likelihood that an individual will employ previously

formed beliefs to inform perceptions, over present evidence available to them (Das and Teng, 1999).

5.6 Methodological Considerations and Study Limitations

The results from the CON group demonstrate a significant effect of the exercise protocol on markers of damage, demonstrating a negative effect of EIMD on performance. These results support the findings that downhill running protocols are effective in inducing muscle damage (Lin et al., 2009; Eston et al., 2006; Braun and Dutto, 2003). Chen et al., (2004) reported that downhill running can induce muscle damage somewhat comparable to damage that occurs post marathon run, additionally, the innate action of running makes it a popular method of inducing damage within a laboratory setting (Lin et al., 2009). Nevertheless, inducing damage within a laboratory setting lacks external validity, when applying the results in a field scenario. Laboratory damage protocols are standardised to ensure the same level of damage across groups. However, this would not necessarily be the case within the field. Individuals performing in a team may experience varying degrees of damage, dependent upon the intensity of the activity and their role within the team. Therefore, when considering the effect of recovery interventions, future research should consider the application in a field experiment to improve ecological validity.

A large effect size was not found, necessary to detect a significant interaction effect of group x time, suggesting that the present study may be underpowered and susceptible to error. Type 1 error occurs when a false positive is reported, falsely rejecting the null hypothesis. Alternatively, a type 2 error occurs when analysis of results indicates a false negative, where the null hypothesis is not rejected and occurs when sample size is not adequate (Banerjee et al., 2009). The present study may be subject to type 2 error as a result of the lack of effect size. Although, a power calculation was computed to determine sample size, due to time

constraints and high rate of participant drop out, the recommended sample size of 28 was not achieved. Future research should consider recruiting more participants, as the lack of significant effect size and statistical significant interaction effect throughout the study may simply be due to the sample size.

The results presented in this study represent percentage change in performance. Therefore, it is necessary to consider what values are considered clinically significant or meaningful to coaches and athletes. The results presented demonstrate small percentage changes in performance post muscle damaging exercise, that at times are <1 %. Nevertheless, previous literature has suggested that in high level athletic competition, the difference between first and last position can be as little as 1 % (Hopkins, Hawley and Burke, 1999; Currell and Jeukendrup, 2008; Allen and Procter, 2016). Therefore, the smallest attenuation of damage that results in improved performance is important to coaches and athletes. However, it remains crucial to consider that this percentage change in performance alongside test retest reliability of the marker of performance.

The test-retest reliability of data obtained in the present study, measured between familiarisation and pre-trials should also be considered. If the CV of marker of damage between familiarisation and pre-trials is greater than the percentage attenuation of damage, then it may be possible that the change is a result of individual variation in performance (Atkinson and Neville, 2001) or measurement error (Page, 2014) and not a result of the intervention. When considering the data from the present study, the results from the performance of MVIC should be interpreted to coaches and athletes with caution due to the test re-test reliability and standard deviation reported for the marker. Additionally, due to the small changes in left and right leg limb circumference and cross sectional area over time, despite the good test re-test reliability of the measure, in some instances it is possible that the

changes reported are a result of measurement error, over the effect of an intervention. Therefore the data should be carefully considered before recommending a recovery intervention to a coach or recreationally active individual.

The population of the present study considered themselves to be recreationally active males, a commonly used population within the literature (Howatson, Goodall and van Someren, 2009; Jakeman, Macrea and Eston, 2009; Stacey et al., 2010). Participants were recruited through a volunteer sampling method. Individuals were asked to state when resistance training was last performed. However, it is possible that participants may not have been honest at this point, as resistance trained males were excluded from the study. Additionally, little inference of the findings can be made to endurance trained or resistance trained individuals, as this was not the sample population. If repeated in endurance or strength trained populations, the findings may differ, due to the differences in physiological make up, training focus and outcome measures. Despite differences in training status, it is necessary for resistance and endurance trained athletes to incorporate recovery into their training programmes.

Crowe et al. (2007) reported a reduction in peak power and total work following a CWI protocol in strength trained males and females, additionally, Paddon-Jones and Quigley (1997) reported no significant effect of CWI on measures of MVIC, arm volume and muscle soreness compared to a control group. These findings may suggest little effect of CWI protocols on resistance trained individuals. However, the effect of CWI on resistance trained males is somewhat equivocal. Vaile et al. (2008) reported an increase in squat MVIC, and reductions in swelling and CK compared to a control group. Similarly, the evidence surrounding the effect of CWI on performance of endurance trained individuals is equivocal. Stanley et al. (2012) reported an improvement in perceptions of recovery and an attenuation of perceptions of pain, additionally, Vaile et al. (2008) reported an increase in sprint

performance compared to a control group. Nevertheless, in a further study a significant increase in subsequent time trial performance compared to an active recovery protocol was present (Vaile et al. 2008; Vaile et al., 2011). The equivocal findings presented in this section demonstrate the difference in response of endurance trained and resistance trained athletes to immersion protocols. Currently, the findings of this study can only be applied to recreationally active populations, therefore, future research should investigate the effects of a TSI protocol within different athletic populations.

Throughout the study a learning effect of the performance of MVIC using the Kin Com was observed. A significant reduction in performance was present in the CON group for CMJ post muscle damaging exercise, suggesting a performance deficit as a result of EIMD. However, this effect was not present during analysis of MVIC. Research has suggested that the ICC values of previous users of an isokinetic dynamometer are lower than those of new users (Meldrum et al., 2009). Participants recruited in the present study varied in experience using the isokinetic dynamometer, therefore, the measure was susceptible to learning effect. In order for a measurement of MVIC to be an accurate marker of damage, future studies should consider a longer familiarisation period to eliminate the implications of this observed learning effect.

Due to practicality and logistical barriers, the maximum depth of immersion that could be achieved was 1.20 m. Therefore, individual participants, although immersed to the same depth, experienced different levels of hydrostatic pressure at the site of damage due to differences in anthropometric measures of limb length. Limb length was reported to establish the pressure exerted for each individual, however, in order to counteract the potential effects of the differing levels of hydrostatic pressure, researchers should consider altering depth of immersion dependent on the anthropometrical measures of individuals in order to standardise

the hydrostatic pressure acting on each individual. The range of hydrostatic pressure exerted on the body was from 89 ± 2 mmHg to 113 ± 4 mmHg. Leeder et al. (2015) reviewed the effects of a seated an standing immersion protocol that exerted pressure from ~40 mmHg during seated immersion and ~111 mmHg during standing immersion. Leeder and co-workers reported no benefit of a CWI protocol in a seated or standing position on recovery. The lack of significance between groups in the present study may be a due to inadequate pressure changes between groups. Therefore, additional methods of increasing pressure may be necessary to further isolate the effects of hydrostatic pressure and temperature. Depths greater than 1.20 m should be considered and compared to seated immersion in research that further investigates the effect of water temperature and hydrostatic pressure on the body. For research purposes, it would be necessary to understand the effect of the maximum immersion depth on recovery, however, this may not be practical to apply in the field. Additionally, the present study utilised a 30% saline immersion, the maximum solubility of NaCl in 35°C water is 0.36 $kg.L^{-1}$. Therefore, the effect of a solution of 36 % salinity should be conducted to investigate any additional benefit. Although, it should be considered that this may not be practical to apply in the field due to issues with dissolving such quantities of salt as some effort was required in order to dissolve a 30 % saline solution. Additionally, it should be noted that throughout the immersion protocol it is possible that a percentage of salt, that was not sufficiently dissolved, may have dropped out of solution, reducing the pressure acting on the site of damage. Nevertheless, the author was satisfied that the solution was adequately saturated with salt, as it would appear that minimal quantities of salt were present once the bath had been drained.

Another consideration is that the literature has suggested that greater levels of adipose tissue at the site of damage may impact the effectiveness of CWI due to the effect on the rate of

intramuscular cooling at the site (Myrer and Meason, 2001). The researchers obtained a skin fold calliper measures at the site of the thigh, in order to account for the level of adipose tissue at the site of damage, this should be considered when interpreting the effectiveness of the recovery strategy. The greater the level of adipose tissue, the longer time required for cooling to be effective (Myrer et al., 2001; Goodall and Howatson, 2009). Table 2 illustrates that the CWI group reported the greatest level of adipose tissue at the site of damage. Nevertheless, CWI demonstrated an overall positive effect on recovery, suggesting that the CWI protocol was of sufficient duration and temperature to stimulate recovery. However, Goodall and Howatson (2009) reported that individuals reporting skin folds of 20 mm or less require 20 min of cooling, this may suggest that a longer duration of CWI may have had a greater effect on recovery. It would be interesting to report the effect of a TSI protocol on those who have a high quantity of adipose tissue, in instances where the mass of adipose tissue may be considered to have a detrimental effect on the rate of intra-muscular cooling that is necessary for CWI to be effective.

5.7 Hormesis

The present study has identified a new method of immersion that does not appear to have a detrimental effect on recovery. However, further research would be necessary to consider the implications of a TSI protocol on hormesis. As defined in the literature review section 2.1.4, hormesis in an important concept to consider when employing an intervention to successfully attenuate the symptoms of damage, whilst minimising the detriment to adaptation. Although the results of the present study, do not consider the effect of TSI, TWI and CWI on adaptation, they do present interesting findings of CK response following a TSI protocol. However, it remains unclear whether this response was a direct result of the TSI protocol or due to higher CK response within the individuals. Nevertheless, it would be interesting to

investigate the effects of a TSI protocol on adaptation and recovery, as results from the present study may stimulate speculation surrounding the effect of this increased CK response, and how this may impact on hormesis. Additionally, it is necessary to research periodisation of immersion protocols to promote adaptation. The conflicting data surrounding the effect of CWI on adaptation would indicate a need to further understand the optimal time to implement interventions during an athlete's training programme and consider perdiodisation of TWI and TSI protocols and the effect on adaptation.

5.8 Future Research Considerations and Practical Applications

To the best of the authors knowledge, this study is the first of its kind to employ a saline immersion protocol, in an attempt to isolate the effects of hydrostatic pressure and temperature to establish the effect on recovery. Therefore, there are still many questions that should be addressed as a result of this research. At this time, no recommendations surrounding a saline immersion protocol as a recovery protocol can be made as there is not enough evidence to suggest its effectiveness and no insight is provided into the effect of a saline immersion protocol on training adaptation. Future research should continue to address saline immersion as a potential recovery strategy, however, research could consider: changing the salinity of the solution, the effect of a cold saline immersion for increased interaction between cold and hydrostatic pressure, the effect of a saline immersion protocol on resistance trained and endurance trained athletes and the effects of a saline immersion protocol on training adaptation.

Analysis of the results of the present study, although lacking in statistical significance, pose some interesting findings. Of particular note, TWI and TSI protocols do not appear to have a detrimental effect on performance. Despite reductions in performance being present in the TWI and TSI groups, the mean result would suggest that a TWI and TSI protocol may be

more effective than no intervention. This may be worthwhile for coaches and athletes to consider dependent when upon their preference in recovery intervention or tolerance of a CWI protocol. However, the effect of a TSI protocol on adaptation is yet to be understood. Nevertheless, this could be of use to athletes who are reluctant to employ interventions as they believe they have to be subjected to CWI protocols in order to benefit. Athletes who suffer from cold intolerances, or individuals who do not respond well to CWI may avoid employing an intervention at all. The present study may suggest that these individuals may benefit from employing a TSI or TWI protocol over no intervention. Nevertheless, further investigation is required to gain further understanding of the action of hydrostatic pressure.

CHAPTER SIX: CONCLUSION

Although the findings of the study are not conclusive, it begins to address the questions of the mechanical actions of water immersion. Initial observations indicate a non-significant trend that may suggest that a CWI protocol is more effective than a TSI protocol, and possibly that the effect of temperature may be greater than that of hydrostatic pressure. Nevertheless, due to the lack of statistical significance between the groups this would require further investigation. Subsequently, the present study accepted the null hypothesis. Furthermore, it should be considered that a CWI protocol stimulates both the effect of temperature and hydrostatic pressure. Therefore, without further isolation of the mechanisms of immersion such as a cold saline, the current findings are inconclusive. Nonetheless, this study has suggested another potential recovery strategy for athletes, although it is an intervention that would require further investigation into the effects of TSI on different populations and the impact of a TSI protocol on training adaptation before it can be recommended as a recovery strategy.

While no primary mechanical action for the effectiveness of water immersion can be inferred at this point, the findings can contribute to the body of research into the effects of water immersion on the body following EIMD. The findings should be considered alongside the previous literature regarding water immersion in order to utilise the most effective recovery protocols that assists recovery from EIMD.

REFERENCES

American College of Sports Medicine (2013). *ACSM's Guidelines for Exercise Testing and Prescription*. 9th ed. New York: Lippincott Williams and Wilkins.

Allen, D. G. (2001). Eccentric muscle damage: mechanisms of early reduction of force. *Acta Physiologica Scandanavica.* 171 (3), pp. 311–319.

Allen, K., and Procter, T. (2016). Winning margins in British Thoroughbred Racehorses. *Comparative Exercise Physiology*. 12(2), 63-66. DOI: 10.3920/CEP16001

Al Haddad, H., Laursen, P, B., Chollet, D., Lemaitre, F., Ahmaidi, S. and Buchheit, M. (2010) Effect of cold or thermoneutral water immersion on post-exercise heart rate recovery and heart rate variability indices. *Autonomic Neuroscience: Basic and Clinical*. 156, pp. 111-116.

Armstrong, R. B. (1984) Mechanisms of exercise-induced delayed onset muscular soreness: a brief review. *Medicine and Science in Sport and Exercise.* 16 (6), pp. 529-538.

Armstrong, R. B., Warren, G. L. and Warren, J. A. (1991) Mechanisms of exercise induced muscle fibre injury. *Sports Medicine*. 12 (3), pp. 184-207.

Ascensao, A., Leite, M., Rebelo, A. N., Magalhaes, S. and Magalhaes, J. (2011) Effects of cold water immersion on the recovery of physical performance and muscle damage following a one- off soccer match. *Journal of Sport Sciences.* 29 (3), pp. 217-225.

Bailey, D. M., Erith, S. J., Griffin, P. J., Dowson, A., Brewer, D. S., Gant, N. and Williams, C. (2007) Influence of cold-water immersion on indices of muscle damage following prolonged intermittent shuttle running. *Journal of Sport Sciences*. 25 (11), pp. 1163-1170.

Baird, M. F., Graham, S. M., Baker, J. S. and Bickerstaff, G. E. (2012) Creatine- kinase and exercise related muscle damage: Implications for muscle performance and recovery. *Journal of Nutrition and Metabolism*. 2012, pp. 1-13.

Baldwin, L. A. (2003) Use of non-steroidal anti-inflammatory drugs following exercise-induced muscle injury. *Sports Medicine*. 33 (3), pp. 177-185.

Banerjee, A., Chitnis, U. B., Jadhav, S. L., Bhawalkar, J. S. and Chaudhury, S. (2009) Hypothesis testing, type I and type II errors. *Industrial Psychiatry Journal*. 18 (2), pp. 127-131.

Banfi, G., Lombardi, G., Colombini, A. and Melegati, G. (2010) Whole-body cryotherapy in athletes. *Sports Medicine*. 40 (6), pp. 509-517.

Baoge, I., Van Den Steen, E., Rimbaut, S., Philips, N., Witvrouw, E., Almqvist, K. F., Vanderstraeten, G. and Vanden Bossche, L.C. (2012) Treatment of skeletal muscle injury: A review. *International Scholarly Research Network.* 2012, pp. 1-7.

Barnett, A. (2006) Using recovery modalities between training sessions in elite athletes. Does it help? *Sports medicine.* 36 (9), pp. 781-796.

Barton, B. E. (1997) IL-6: Insights into novel biological activities. *Clinical Immunology and Immunopathology.* 85, pp. 16-20.

Batterham, A. M. and Hopkins, W. G. (2005) Making meaningful inferences about magnitudes. *International Journal of Sports Physiology and Performance.* 1 (1), pp. 50-57.

Becker, B.E. and Cole, A.J., 1998. Aquatic rehabilitation. *Rehabilitation medicine: principles and practice, 3rd ed. Philadelphia: Lippincott–Raven*. pp. 897-901.

Becker, B. E., Hildenbrand, K., Whitcomb, R. K. and Sanders, J. P. (2009) Biophysiologic effects of warm water immersion. *International Journal of Aquatic Research and Education.* 3, pp. 24-37.

Becker, B. (2009) Aquatic therapy: Scientific foundations and clinical rehabilitation applications. *PM & R.* 1 (9), pp. 859- 872.

Balnave, C. D. & Allen, D. G. (1995). Intracellular calcium and force in single mouse muscle fibres following repeated contractions with stretch. *Journal of Physiology.* 488, pp. 25–36.

Beltman, J. G. M., van der Vliet. M. R., Sargeant, A. J. and de Haan, A. (2004) Metabolic cost of lengthening, isometric and shortening contractions in maximally stimulated rat skeletal muscle. *Acta Physiologica Scandinavic.* 182, pp. 179-187.

Bergel, R. Hydrotherapy: Hot-Cold-Wet-Dry Spa Treatments. pp. 1-9 http://www.sld.cu/galerias/pdf/sitios/rehabilitacion-bal/hydrotherapy.pdf.

Best, T. M., Gharaibeh, B., Huard, J. (2013) Stem cells, angiogenesis and muscle healing: a potential role in massage therapies. *British Journal of Sports medicine.* 47, pp. 556-560.

Bieuzen, F., Bleakley, C. M. and Costello, J. T. (2013) Contrast water therapy and exercise induced muscle damage: A systematic review and meta-analysis. *PLoS ONE.* 8 (4), pp. e62356.

Bieuzen, F., Brisswalter, J., Easthope, C., Vercruyssen, F., Bernard, T. and Hausswirth, C. (2014) Effect of wearing compression stockings on recovery after mild exercise induced muscle damage. *International Journal of Sports Physiology and Performance.* 9, pp. 256-264.

Bird, S., (2011) Post exercise recovery. *The Australian Orienteer.* pp. 10-13

Bleakley, C. and Davison, G. W. (2010) What is the biochemical and physiological rationale for using cold-water immersion in sports recovery? A systematic review. *British Journal of Sports Medicine*. 44, pp. 179-187.

Boer, J., Schothorst, A., Boom, B., Hermans, J. and Suurmond, D. (1982) Influence of water and salt solutions on UVB irradiation of normal skin and psoriasis. *Archives of Dermatological Research*. 273, pp. 247-259.

Bosak, A., Bishop, P. and Green, J. (2009) Impact of cold water immersion on 5 km racing performance. *The Sports Journal.* 12 (2).

Bove, A. A. (2002) Medical disorders related to diving. *Journal of Intensive Care Medicine.* 17 (2), pp. 75-86.

Braun, W. A. and Dutton, D. J. (2003) The effects of a single bout of downhill running and ensuring delayed onset muscle soreness on running economy performed 48 h later. *European Journal of Applied Physiology.* 90, pp. 29-34.

Braun, W. A. and Paulson, S. (2012) The effects of downhill running bout on running economy. *Research in Sports Medicine*. 20, pp. 274-285.

Bridgeman, L. A., McGuigan, M. R. and Gill, N. D. (2015) Eccentric exercise, exercise induced muscle damage and the repeated bout effect: A brief review. *Journal of Australian Strength and Conditioning.* 23 (3), pp. 74-74.

Brophey-Williams, N., Landers, G. and Wallman, K. (2011) Effect of immediate and delayed cold water immersion after high intensity exercise session on subsequent run performance. *Journal of Sport Science and Medicine*. 10, pp. 665-670.

Burgess, T. L. and Lambert, M, I. (2010) The effects of training, muscle damage and fatigue on running economy. *International Sports Medicine Journal.* 11 (4), pp. 363-379.

Burt, D. G., Lamb, K., Nicholas, C. and Twist, C. (2014) Effects of exercise-induced muscle damage on resting metabolic rate, sub-maximal running and post-exercise oxygen consumption. *European Journal of Sport Science.* 14 (4), pp. 337-344.

Byrne, C. and Eston R. (2002b) The effect of exercise-induced muscle damage on isometric and dynamic knee extensor strength and vertical jump performance. *Journal of Sports Science.* 20, pp. 417-425.

Byrne, C., Twist, C. and Eston, R. (2004) Neuromuscular function after exercise-induced muscle damage: Theoretical and applied interventions. *Sports Medicine*. 34 (1), pp. 49-69.

Calle, M, C. and Fernandez, M. L. (2010) Effects of resistance training on the inflammatory response. *Nutrition, Research and Practice.* 4 (4), pp. 259-269.

Camf, J., Guerra, D., Ugena, B., Sergura, J. and De La Torre, R. (1991) Effect of subject expectancy on the THC intoxication and disposition from smoked hashish cigarettes. *Pharmacology, Biochemistry and Behaviour*. 40, pp. 115-119.

Casa, D. J., Clarkson, P. M. and Roberts, W. O. (2005) American college of sports medicine roundtable on hydration and physical activity: consensus and statements. *Current Sports Medicine Reports*. 4, pp. 115-127.

Chen T.C. and Nosaka K (2006) Effects of number of eccentric muscle actions on first and second bouts of eccentric exercise of the elbow flexors. *Journal of Science and Medicine in Sport.* 9, pp. 57–66.

Chen, H-L., Nosaka, K., Pearce, A. J. and Chen, T. C. (2012) Two maximal isometric contractions attenuate the magnitude of eccentric exercise induced muscle damage. *Applied Physiology, Nutrition and Metabolism.* 37, pp. 680-689.

Cheung, K., Hume, P. and Maxwell, L. (2003) Delayed onset muscle soreness: treatment strategies and performance factors. *Sports Medicine*. 33 (2), pp. 145-164.

Clarkson, P. M. and Sayers, S. P. (1999) Etiology of exercise induced muscle damage. *Canadian Journal of Applied Physiology.* 24 (3), pp. 234-248.

Clarkson, P. M. and Hubbal, M. J. (2002) Exercise-induced muscle damage in humans. *American Journal of Physical Medicine and Rehabilitation.* 81 (11), pp. 52-69.

Cleak, M. J. and Eston, R. G. (1992) Delayed onset muscle soreness: mechanisms and management. *Journal of Sports Science.* 10 (4), pp. 325-341.

Close, G., Table, N. T., Doran, D. and Maclaren, D. P. (2006) Asorbic acid supplementation does not attenuate post-exercise muscle soreness following muscle-damaging exercise but may delay the recovery process. *British Journal of Nutrition*. 95 (5), pp. 976-981.

Cochrance, D. J. (2004) Alternating hot and cold water immersion for athletic recovery: A review. *Physical Therapy in Sport*. 5, pp. 26-32

Cohen, J. (1969) Statistical power analysis for the behavioural science. New York: Academic Press.

Conceicao, M. S., Libardi, C. A., Chacon-Mikahil, M. P. T., Nogueira, F. R. D., Vechin, F. C., Bonganha, V., Bernardes, C. F., Madruga, V. A. and Cavaglieri, C. R. (2014) Inflammatory responses after different velocities of eccentric exercise. *Isokinetics and Exercise Science*. 22, pp. 77-84.

Connolly, D. A.J., Sayers, S. P. and McHugh, M. P. (2003) Treatment and prevention of delayed onset muscle soreness. *Journal of Strength and Conditioning Research.* 17 (1), pp. 197-208.

Cook, C. J. and Beaven, C. M. (2013) Individual perception of recovery is related to subsequent sprint performance. *British Journal of Sports medicine.* 0, pp. 1-5.

Corbett, J., Barwood, M. J., Lunt, H. C., Milner, A. and Tipton, M, J. (2012) Water immersion as a recovery aid from intermittent shuttle running exercise. *European Journal of Sport Science.* 12 (6), pp. 509-514.

Crowe, M. J., O'Connor, D. and Rudd, D. (2007) Cold water immersion reduces anaerobic performance. *International Journal of Sports Medicine.* 28, pp. 994-998.

Crystal, N. J., Townson, D. H., Cook, S. B. and LaRoche, D. P. (2013) Effect of cryotherapy on muscle recovery and inflammation following a bout of damaging exercise. *European Journal of Applied Physiology.* 113, pp. 2577-2586.

Currell, K and Jeukendrup, E. (2008) Validity, Reliability and Sensitivity of Measures of Sporting Performance. *Sports Medicine.* 38 (4), pp. 297-316.

Das, T. K. and Teng, B. S. (1999) Cognitive bias and strategic decision processes: an integrative perspective. *Journal of Management Studies*. 36 (6), pp. 757- 778.

Davies, R. C. (2010) *The effects of exercise induced muscle damage on the human response to dynamic exercise*. PhD Thesis. University of Exeter.

Davis, M. J., Murphy, A. E., Carmichael, M. D. Zielinski, M. R., Groschwitz, C. M., Brown, A. S., Gangeml, D. J., Ghaffar, A. and Mayer, E. P. (2007) Curcumin effects on inflammation

and performance following eccentric exercise-induced muscle damage. *American Journal of Physiology- Regulatory, Intergrative and Comparative Physiology.* 292, pp. R2168- R2173

Durlack, J.A. (2009) How to select, calculate, and interpret effect sizes. *Journal of Pediatric Psychology.* 34, pp. 32-36.

Elias, G. P., Varley, M. C. Wyckelsma, V. L., McKenna, M. J., Minahan, C. L. and Aughey, R. J. (2012) Effects of water immersion on post-training recovery in Australian footballers. *International Journal of Sports Physiology and Performance.* 7, pp. 357-366.

Epstein, M. (1976) Cardiovascular and renal effects of head-out water immersion in man: Application of the model in the assessment of volume homeostasis. *American Heart Association.* 39 (5), pp. 619- 628.

Eston, R., Mickleborough, J. and Baltzopoulos, V. (1995) Eccentric activation and muscle damage: biomechanical and physiological considerations during downhill running. *British Journal of Sports Medicine*. 29 (2), pp. 89-94.

Eston, R. and Peters, D. (1999) Effects of cold water immersion on the symptoms of exercise induced muscle damage. *Journal of Sports Sciences.* 17 (3), pp. 231-238.

Eston, R. G., Lemmey, A. B., McHugh, P., Byrne, C. and Walsh, S. E. (2000) Effect of stride length on symptoms of exercise-induced muscle damage during a repeated bout of downhill running. *Scandinavian Journal of Medicine and Science in Sports.* 10, pp. 199-204.

Eston, R., Byrne, C. and Twist, C. (2003) Muscle function after exercise-induced muscle damage: Considerations for athletic performance in children and adults. *Journal of Exercise Science and Fitness.* 1 (2), pp. 85-96.

Frangolias, D. D. and Rhodes, E. C. (1996) Metabolic responses and mechanisms during water immersion running and exercise. *Sports Medicine.* 22 (1), pp. 38-53.

Franzini-Armstrong, C. and Jorgensen, A. (1994) Structure and development of the E-C coupling units in skeletal muscle. *Annual review of physiology.* 56, 509-534.

French, D. N., Thompson, K. G., Garland, S. W., Barnes, C. A. Portas, M. D., Hood, P. E. and Wilkes, G. (2008) The effects of contrast bathing and compression therapy on muscular performance. *Medicine and Science in Sports and Exercise.* pp. 1297- 1306.

Friden, J. and Lieber, R. L. (1992) Structural and mechanical basis of exercise-induced muscle injury. *Medicine and Science in Sport and Exercise.* 24 (5), pp. 521-530.

Gabriel, H. and Kindermann, W. (1997) The acute immune response to exercise: What does it mean? *International Journal of Sports Medicine.* 18 (1), pp. S28-S45.

Gagliano M, Corona D, Giuffrida G, et al. (2009) Low-intensity body building exercise induced rhabdomyolysis: a case report. *Cases Journal*. 2 (1) article 7.

Gavin, J. P., Myers, S. D. and Willems, M. E. T. (2015) The effect of glycogen reduction on cardio-respiratory and metabolic responses during downhill running. *European Journal of Applied Physiology*. 115, pp. 1125-1133.

Gill, N. D., Beaven, C. M. and Cook, C. (2006) Effectiveness of post-match recovery strategies in rugby players. *British Journal of Sports Medicine.* 40, pp. 260-263.

Givli, S. (2015) Contraction induced muscle injury: Towards personalized training and recovery programs. *Annals of Biomedical Engineering.* 43 (2), pp. 388-403.

Glasgow, P. D., Ferris, R. and Bleakley, C. M. (2014). Cold water immersion in the management of delayed-onset muscle soreness: Is dose important? A randomised controlled trial. *Physical Therapy in Sport.* pp. 1-6.

Gleeson, M., Blannin, A. K., Walsh, N. P., Field, C. N. E. and Pritchard, J. C. (1998) Effect of exercise induced muscle damage on the blood lactate response to incremental exercise in humans. *European Journal of Applied Physiology.* 77, pp. 292-295.

Golden, C. L. and Dudley, G. A. (1992) Strength after bout of eccentric or concentric actions. *Medicine and Science in Sports and Exercise.* 24 (8), pp. 926–33

Goodall, S. and Howatson, G. (2008) The effects of multiple cold water immersions on indices of muscle damage. *Journal of Sports Science and Medicine.* 7, pp. 235-241.

Greenawalt, T.J., Kidder, A. J., Seiner, E. B. and Cacolice, P. A. (2004) The effectiveness of cold water immersion in reducing delayed onset muscle soreness in collegiate athletes. *Conference Paper.*

Halson, S. L., Quod, M. J., Martin, D. T., Gardner, A. S., Ebert, T. R. and Laursen, P. B. (2008) Physiological responses to cold water immersion following cycling in the heat. *Journal of Sports Physiology and Performance*. 3, pp. 331-346.

Hausswirth, C., Louis, J., Bieuzen, F., Pournot, H., Fournier, J., Filliard, J., Brisswalter, J. (2011) Effects of whole body cryotherapy vs. far-infrared vs. passive modalities on recovery from exercise-induced muscle damage in highly trained runners. *PLoS ONE.* 6 (12), pp. e277749

Hayter, K. J., Doma, K., Schumann, M. and Deakin, G. B. (2016) The comparison of cold-water immersion and cold air therapy on maximal cycling performance and recovery markers following strength exercises. *Peer J.* 4 (e1841), pp. 1-17.

Heyman, E., De Geus, B., Mertens, I. and Meeusen, R. (2008) Effects of four recovery methods on repeated maximal rock climbing performance. *Medicine and Science in Sports and Exercise.* pp. 1303- 1310.

Higgins, T. R., Cameron, M. L. and Climstein, M. (2013) Acute response to hydrotherapy after a simulated game of rugby. *Journal of Strength and Conditioning Research.* 27 (10), pp. 2851-2860.

Hill, J., Howatson, G., van Someren, K., Leeder, J. and Pedlar, C. (2014) Compression garments and recovery from exercise-induced muscle damage: a meta-analysis. *British Journal of Sports Medicine*. 48, pp. 1340-1346.

Hirose, L., Nosaka, K., Newton, M., Laveder, A., Kano, M., Peake, J. and Suzuki, K. (2004) Changes in inflammatory mediators following eccentric exercise of the elbow flexors. *Exercise Immunology Review*. pp. 75-90.

Hoffman-Goetz, I., Thorne, R., Randall Simposon, J. A. and Arumugam, Y. (1989) Exercise stress alters murine lymphocyte subset distribution in spleen, lymph nodes and thymus. *Clinical and Experimental Immunology*. 76, pp. 307-310.

Hohenauer, E., Taeymans, J., Baeyens, J. P., Clarys, P. and Clijesen, R. (2015) The effect of post exercise cryotherapy on recovery characteristics: A systematic review and meta-analysis. *PLoS ONE.* 10 (9), pp. 1-22.

Hopkins, W., Hawley, J and Burke, L. (1999) Design and analysis of research on sport performance enhancement. *Medicine and Science in Sport and Exercise*. 31 (3), pp. 472-485.

Hopkins, W. G. (2000) Measures of reliability in sports medicine and science. *Sports Medicine*. 30 (1), pp. 1-15.

Hopkins, W. G. (2000) Reliability from consecutive pairs of trials (excel spreadsheet). *A New View of Statistics: Sportscience.org, Internet Society for Sportscience.* www.sportsci.org/resource/stats/xrely.xls.

Hopkins, W. G. (2002) Probabilities of clinical or practical significance. *SportScience 6.* pp. 1-2.

Horder, M., Jorgensen, P. J., Hafkenscheid, J. C., Carstensen, C. A., Bachmann, C., Bauer, K., Neuwald, C., Rosalki, S. B., Foo, A. Y. and Vogt, W. (1991) Creatine Kinase determination: a European evaluation of the creatine kinase determination in serum, plasma and whole blood with the reflotron system. *European Journal of Clinical Chemistry and Clinical Biochemistry*. 29 (10), pp. 691-696.

Hortobágyi, T., Money, J., Zeng, D. and Dohm, L. (2002) Muscle Adaptations to 7 Days of Exercise in Young and Older Humans: Eccentric Overload versus Standard Resistive Training. *Journal of aging and physical activity.* 10 (3), pp. 290-305.

Hough, T. (1902) Ergographic studies in muscular soreness. *American Journal of Physiology.* 7, pp. 76-92.

Howatson, G., van Someren, K. and Hortobagyi, T. (2007) Repeated bout effect after maximal eccentric exercise. *International Journal of Sports Medicine.* 28, pp. 557-563.

Howatson, G. and van Someren, K. A. (2008) The prevention and treatment of exercise induced muscle damage. *Sports Medicine.* 38 (6), pp. 483-503.

Howatson, G., Goodall, S. and van Someren, K. A. (2009) The influence of cold water immersions on adaptation following a single bout of damaging exercise. *European Journal of Applied Physiology.* 105, pp. 615-621.

Howatson, G., Hoad, M., Goodall, S., Tallent, J., Bell, P. G. and French, D. N. (2012) Exercise-induced muscle damage is reduced in resistance-trained males by branched chain amino acids: a randomized, double-blind, placebo controlled study. *Journal of the International Society of Sports Nutrition.* 9 (20), pp. 1-7.

Howatson, G., Leeder, J. and van Someren, K. (2016) The BASES expert statement on athletic recovery strategies. *The Sport and Exercise Scientist*. 48, pp. 6-7.

Huerta-Alardin, A., Varon, J. and Marik, P. E. (2005) Bench-to-bedside review: Rhabdomyolysis- and overview for clinicians. *Journal of Critical Care.* 9 (2), pp. 158-169.

Hyldahl, R. D. and Hubal, M. J. (2014) Lengthening our perspective: morphological, cellular and molecular responses to eccentric exercise. *Muscle and Nerve*. 49, pp. 155-170.

Ingram, J., Dawson, B., Goodman, C., Wallman, K. and Beilby, J. (2009) Effect of water immersion methods on post-exercise recovery from simulated team sport exercise. *Journal of Science and Medicine in Sport.* pp. 417-421.

Ihsan, M., Watson, G., Choo, H. C., Lewandowski, P., Papazzo, A., Cameron- Smith, D. and Abbiss C. R. (2014) Post-exercise muscle cooling enhances gene expression of PGC-1α. *Medicine and Science in Sports and Exercise.* 46 (10), pp. 1900–1907. doi:10.1249/MSS.0000000000000308

Ihsan M, Markworth J. F, Watson, G., Choo, C. H., Govus, A., Pham, T., Hickey, A. J., Cameron-Smith, D. and Abbiss, C. R. (2015) Regular post-exercise cooling enhances mitochondrial biogenesis through AMPK and p38 MAPK in human skeletal muscle. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology.* doi:10.1152/ajpregu.00031.2015

Jakeman, J., Macrae, R. and Eston, R. (2009) A single 10-min bout of cold water immersion therapy after strenuous plyometric exercise has no beneficial effect on recovery from the symptoms of exercise induced muscle damage. *Ergonomics*. 52 (4), pp. 456-460.

Jakeman, J. R., Byrne, C. and Eston, R. G. (2010) Lower limb compression garments improves recovery from exercise-induced muscle damage in young, active females. *European Journal of Applied Physiology.* 109 (6), pp. 1137-1144.

Jarvinen, T. A., Jarvinen, T. L. N., Kaarianinen, M., Kalimo, H. and Jarvinen, M. (2005) Muscle Injuries: Biology and Treatment. *The American Journal of Sports Medicine.* 33 (5), pp. 745-764.

Jones, A. M. and Doust, J. H. (1996) A 1% treadmill grade most accurately reflects the energetic cost of outdoor running. *Journal of Sports Sciences.* 14, pp. 321-327.

Jones, A. and Carter, H. (2000) The effect of endurance training on parameters of aerobic fitness. *Sports Medicine.* 29 (6), pp. 373-386.

Joo, C. H., Allan, R., Drust, B., Close, G. L., Jeong, T. S., Bartlett, J. D., Mawhinney, C., Louhelainen, J., Morton, J. P. and Gregson, W. (2016) Passive and post-exercise cold-water immersion augments PGC-1α and VEGF expression in human skeletal muscle. *European Journal of Applied Physiology*. DOI 10.1007/s00421-016-3480-1

Kaczmarek, M., Mucha, D. and Jarawka, N. (2013) Cold water immersion as a post-exercise recovery strategy. *Medicina Sportiva*. 17 (1), pp. 35-39.

Kanda, K., Sugama, K., Hayashida, H., Sakuma, J., Kawakami, Y., Miura, S., Yoshioka, H., Mori, Y. and Suzuki, K. (2013) Eccentric exercise-induced delayed onset muscle soreness and changes in markers of muscle damage and inflammation. *Exercise Immunology Review.* 19, pp. 72-85.

Kuligowski, L. A., Lephart, S. M., Giannantonio, F. P. and Blanc, R. O. (1998) Effects of whirlpool therapy on the signs and symptoms of delayed onset muscle soreness. *Journal of Athletic Training.* 33 (3), pp. 222-228.

Lakens, D. (2013) Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for *t*-tests and ANOVAs. *Frontiers in Psychology*. 4, pp. 1-12.

Lamb, G. D. (2009) Mechanisms of excitation-contraction uncoupling relevant to activity-induced muscle fatigue. *Applied Physiology, Nutrition, and Metabolism.* 34 (3), pp. 368-372.

Lane, K., Worsley, D. and McKenzie, D. (2005) Exercise and the lymphatic system: Implications for breast cancer survivors. *Sports Medicine.* 35 (6), pp. 461-471.

Lastayo, P, Reich,T, Urquhart, M, Hoppeler, H. and Lindstedt, S. (1999) Chronic eccentric exercise: improvements in muscle strength can occur with little demand for oxygen. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology.* 276 (2), pp R611-R615.

LaStayo, P. C., Woolf, J. M., Lewek, M. D., Snyder-Mackler, L., Reich, T. and Lindstedt, S. L. (2003) Eccentric muscle contractions: Their contribution to injury, prevention, rehabilitation and sport. *Journal of Orthopaedic & Sports Physical Therapy.* 33, pp. 557-571.

Lee, J., Goldfarb, A. H., Rescino, M. H., Hedge, S., Patrick, S. and Apperson, K. (2001) Eccentric exercise effect on blood oxidative-stress markers and delayed onset muscle soreness. *Medicine and Science in Sport and Exercise*. 34 (3), pp. 443-448.

Leeder, J., Gissane, C., van Someren, K., Gregson, W. and Howatson, G. (2012) Cold water immersion and recovery from strenuous exercise: A meta- analysis. *British Journal of Sports Medicine.* 46, pp. 233-240.

Leeder, J., van Someren, K. A., Bell, P. G., Spence, J. R., Jewell, A. P., Gaze, D. and Howatson, G. (2015) Effects of seated and standing cold water immersion on recovery from repeated sprinting. *Journal of Sport Sciences*. 33 (15), pp. 1544-1552.

Leveritt, M., Abernethy, P. J., Barry, B. K. and Logan, P. A. (1999) Concurrent Strength and Endurance training. *Sports Medicine.* 28 (6), pp. 413-427.

Lieber, R. L. and Friden, J. (2002) Morphological and mechanical basis of delayed-onset muscle soreness. *Journal of the American Academy of Orthopaedic Surgeons.*10, pp. 67-73.

Lima, K. M. da Matta, T. T. and de Oliveira, L. F. (2012) Reliability of the rectus femoris muscle cross-sectional area measurements by ultrasonography. *Clinical Physiology and Functional Imaging.* 32, pp. 221- 226.

Lin, M-J., Chen, T. C., Chen, H-L., Wu, C-J., Tseng, W-C. (2009) Effects of gradient variations on physiological responses to a 30 minute run. *Journal of Exercise Science & Fitness.* 7 (2), pp. 85-90.

Linstedt, S. L., LaStayo, P. C. and Reich, T. E. (2001) When active muscles lengthen: properties and concequences of eccentric contractions. *News in Physiological Sciences.* 16, pp. 256-261.

Machado, A. F., Ferreira, P. H., Micheletti, J. K., de Almeida, A. C., Lemes, I. R., Vanderlei, F. M., Junior, J. N. and Pastre, C. M. (2016) Can water temperature and immersion time influence the effect of cold water immersion on muscle soreness? A systematic review and meta-analysis. *Sports Medicine.* 53, 503. doi:10.1007/s40279-015-0431-7.

Mair, J., Mayr, M., Muller, E., Koller, A., Haird, C., Artnerdworzak, E., Calzolari, C., Laurie, C. and Puschendorf, B. (1995) Rapid adaptation to eccentric exercise-induced muscle damage. *International Journal of Sports Medicine*. 16 (6), pp. 352-356.

Malm, C. (2001) Exercise-induced muscle damage and inflammation: fact or fiction? *Acta Physiologica Scandinavica*. 171, pp. 233-239.

Martinez, I. (1995-2016) Properties of some particular solutions. pp.1-29 http://webserver.dmt.upm.es/~isidoro/bk3/c07sol/Solution%20properties.pdf

Matz, H., Orion, E. and Wolf, R. (2003) Balneotherapy in dermatology. *Dermatologic Therapy.* 16, pp. 132-140.

McCutcheon, S. C., Martin, J. L. and Barnwell, T. O. (1993) Water density as a function of temperature and concentration. Water quality in maidment, D. R. New York, NY. McGraw-Hill, pp 11.3.

McGinley, C., Shafat, A. and Donnelly, A. E. (2009) Does antioxidant vitamin supplementation protect against muscle damage. *Sports Medicine.* 39 (12), pp. 1011-1032.

McHugh, M., Connolly, D. J., Eston, R. G. and Gleim, G. W. (1999) Exercise induced muscle damage and potential mechanisms for the repeated bout effect. *Sports Medicine.* 27 (3), pp. 157-170.

McHugh, M., Connolly, D. J., Eston, R. G., Kremenic, I. J., Nicholas, S. J. and Gleim, G. W. (1999) The role of passive muscle stiffness in symptoms of exercise-induced muscle damage. *The American Journal of Sports Medicine*. 27 (5), pp. 594- 599.

McHugh, M. P. (2003) Recent advances in the understanding of the repeated bout effect: the protective effect against muscle damage from a single bout of eccentric exercise. *Scandinavian Journal of Medicine and Science in Sport*. 13, pp. 88-97.

McKune, A. J., Semple, S. J. and Perters-Futre, E. M. (2012) Acute exercise-induced muscle injury. *Biology of Sport*. 29, pp. 3-10.

Meldrum, D., Cahalane, E., Keogan, F. and Hardiman, O. (2002) Maximum voluntary isometric contraction: Investigation of reliability and learning effect. *Amyotrophic Lateral Sclerosis*. 4 (1), pp. 36-44.

Merrick, M. A., Jutte, L. S. and Smith, M. E. (2003) Cold modalities with different thermodynamic properties produce different surface and intramuscular temperatures. *Journal of Athletic Training*. 38 (1), pp. 28-33.

Minahan, C., Joyce, S., Bulmer, A. C., Cronin, N. and Sabapathy, S. (2015) The influence of estradiol on muscle damage and leg strength after intense eccentric exercise. *European Journal of Applied Physiology*. 115, pp. 1493-1500.

Minett, G., Duffield, R., Kellett, A. and Portus, M. (2012) Effects of mixed-method cooling on recovery of medium-fast bowling performance in hot conditions on consecutive days. *Journal of Sport Sciences*. 30 (13), pp. 1387-1396.

Miyama, M. and Nosaka, K. (2004b) Influence of surface on muscle damage and soreness influence by consecutive drop jumps. *Journal of Strength and Conditioning Research.* 18, pp. 206-211.

Moraska, A. (2005) Sports Massage: A comprehensive review. *Journal of Sports Medicine and Physical Fitness.* 45, pp. 370-380.

Morton, R. H. (2007) Contrast water immersion hastens plasma lactate decrease after intense anaerobic exercise. *Journal of Science and Medicine in Sport.* 10, pp. 467-470.

Morgan, D. L. (1990). New insights into the behavior of muscle during active lengthening. *Biophysics Journal*. 57 (2), pp. 209–221

Morgan, D. L. and Proske, U. (2004) Popping sarcomere hypothesis explains stretch induced muscle damage. *Proceedings of the Australian Physiological and Pharmacological Society.* 34, pp. 19-23.

Moses, A. W., David, M., Goldhammer, E., Tal, A. and Sukenik, S. (2006) The dead sea, a unique natural health resort. *The Israel Medical Association Journal.* 8, pp. 483-488.

Myrer, J William; Myrer, Kimberly A; Measom, Gary J; Fellingham, Gilbert W; Evers, Stacey L. (2001) Muscle temperature is affected by overlying adipose tissue when cryotherapy is administered, *Journal of Athletic Training*. 36 (1) pp. 32-36.

Nakamura, K. Takahashu, H., Shimai, S. and Tanaka, M. (1996) Effects of immersion in tepid bath water on recovery from fatigue in submaximal exercise in man. *Ergonomics*. 39 (2), pp. 257-266.

Newham, D.J. Jones, D.A. and Edwards, R. H. T. (1983) Large delayed plasma creatine kinase changes after stepping exercise. *Muscle and Nerve*. 6, pp. 380-385.

Nieman, D. C. (1994) Exercise, Infection and Immunity. *International Journal of Sports Medicine*. 15 (3), pp. S131- S141.

Nogueira, F. R. D., Conceicao, M. S., Vechin, F. C., Junior, E. M. M., Rodrigues, G. F. C., Fazolin, M. A., Chacon-Mikahil, M. P. T. and Libardi, C. A. (2013) The effect of eccentric contraction velocity on muscle damage: A review. *Isokinetics and Exercise Science*. 21, pp. 1-9.

Nonaka, K., Akiyama, J., Tatsuta, N. and Une, S. (2012) Cold water immersion after downhill running suppresses exercise induced muscle damage in the rat soleus muscle. *Journal of Physical Therapy in Science*. 24, pp. 613-616.

Nosaka, K. and Clarkson, P. M. (1995) Muscle damage following repeated bouts of high force eccentric exercise. *Medicine and Science in Sport and Exercise.* 27 (9), pp. 1263-1269.

Nosaka, K., Sakamoto K, Newton M, Sacco P (2001) The repeated bout effect of reduced-load eccentric exercise on elbow flexor muscle damage. *European Journal of Applied Physiology*. 85, pp. 34–40.

Nosaka, K. and Newton, M. (2002) Concentric or eccentric training effect in eccentric exercise induced muscle damage. *Medicine and Science in Sport and Exercise*. pp. 63- 69.

Ostrowski, K., Rohde, T., Zacho, M., Asp, S. and Pedersen, B. K. (1998) Evidence that interleukin-6 is produced in human skeletal muscle during prolonged running. *Journal of Physiology*. 508, pp. 949-953.

Paddon-Jones, D. and Quigley, B. (1997) Effect of cryotherapy on muscle soreness and strength following eccentric exercise. *International Journal of Sports Medicine*. 18 (8), pp. 588-593.

Page, P. (2014) Beyond statistical significance: Clinical interpretation of rehabilitation research literature. *International Journal of sports physical therapy*. 9 (5), 726-736.

Papadopoulos, S., Jurgens, K. D. and Gros, G. (2000) Protein diffusion in living skeletal muscle fibres: dependence on protein size, fibre type and contraction. *Biophysical Journal*. 79, pp. 2084-2094.

Parati, G., Stergiou, G. S., Asmar, R., Bilo, G., de Leeuw, P., Imai, Y., Kario, K., Lurbe, E., Manolis, A., Mengden, T. and O'Brien, E. (2008) European Society of Hypertension guidelines for blood pressure monitoring at home: a summary report of the Second International Consensus Conference on Home Blood Pressure Monitoring. *Journal of hypertension*, 26 (8), pp. 1505-1526.

Park, K. S., Choi, J. K. and Park, Y. S. (2000) Cardiovascular regulation during water immersion. *Applied Human Science: Journal of Physiological Anthropology.* 18 (6), pp. 233-241.

Park, K. S. and Lee, M. G. (2015) Effects of unaccustomed downhill running on muscle damage, oxidative stress and leukocyte apoptosis. *Journal of Exercise, Nutrition and Biochemistry*. 19 (2), pp. 55-63.

Pasiakos, S. M., Lieberman, H. R. and McLellan, T. M. (2014) Effects of protein supplementation on muscle damage, soreness, and recovery of muscle function and physical performance: A systematic review. *Sports Medicine*. 44 (5), pp. 655-670.

Paulson, G., Mikkelsen, U. R., Raastad, T. and Peake, J. M. (2012) Leucocytes, cytokines and satellite cells: what role do they play in muscle damage and regeneration following eccentric exercise? *Exercise Immunology Review*. 18, pp. 42-97.

Peake, J. M., Suzuki, K., Wilson, G., Hordern, M., Nosaka, K., Mackinnon, L. and J. S. Coombes (2005) Exercise-induced muscle damage, plasma cytokines, and markers of neutrophil activation. *Medicine and science in sports and exercise*. 37 (5), pp. 737-745.

Peake, J. M., Merkworth, J. F., Nosaka, K., Raastad, T., Wadley. G.D., Coffey, V. G. (2015) Modulating exercise induced hormesis: does less equal more? *Journal of Applied Physiology*. 119, pp. 178-189.

Pedersen, B. K. and Toft, A. D. (2000) Effects on lymphocytes and cytokines. *British Journal of Sports Medicine.* 34, pp. 246-251.

Pedersen, B. K. and Hoffman-Goetz, L. (2000) Exercise and the immune system: Regulation, integration and adaptation. *Physiological Reviews*. 80 (3), pp. 1055- 1081.

Pedersen, B. K., Steensberg, A., Fischer, C., Keller, C., Ostrowski, K. and Schjerling, P. (2001) Exercise and cytokines with particular focus on muscle-derived IL-6. *Exercise Immunology Review*. 7, pp. 1-14.

Peiffer, J. J., Abbiss, C. R., Nosaka, K., Peake, J. M. and Laursen, P. B. (2008) Effect of cold water immersion after exercise in the heat on muscle function, body temperatures, and vessel diameter. *Journal of Science and Medicine in Sport*. 12 (1), pp. 91-96.

Peiffer, J. J., Abbis, C. R., Watson, G., Nosaka, K. and Laursen, P. B. (2009). Effect of cold-water immersion duration on body temperature and muscle function. *Journal of Sport Sciences*. 27 (10), pp. 987-993.

Pierrynowski, M. R., Tüdus, P. M. and Plyley, M. J. (1987) Effects of downhill or uphill training prior to a downhill run. *European Journal of Applied Physiology.* 56, pp. 668–72

Plattner, K., Lambert, M. I., Tam, N. and Baumeister, J. B. (2014) The response of cortical alpha activity to pain and neuromuscular changes caused by exercise-induced muscle damage. *Scandinavian Journal of Medicine and Science in Sports.* 24, pp. 166-178.

Pointon, M., Duffield, R., Cannon, J. and Marino, F. E. (2011) Cold application for neuromuscular recovery following intense lower-body exercise. *European Journal of Applied Physiology.* 111, pp. 2977-2986.

Pointon, M. and Duffiled, R. (2012) Cold water immersion recovery after simulated collision sport exercise. *Medicine and Science in Sports and Exercise*. 44 (2), pp. 206-216.

Pournot, H., Bieuzen, F., Duffield, R., Lepretre, P. M., Cozzolino, C. and Hausswirth, C. (2011) Short term effects of various water immersion on recovery from exhaustive intermittent exercise. *European Journal of Applied Physiology.* 111, pp. 1287-1295.

Powers, S. K. and Jackson, M. J. (2008) Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiological Reviews.* 88 (4), pp. 1243-1276.

Prisk, V. and Huard, J. (2003) Muscle injuries and repair: The role of prostaglandins and inflammation. *Histology and Histopathology.* 18, pp. 1243-1256.

Proske, U. and Morgan, D. L. (2001) Muscle damage following eccentric exercise: mechanism, mechanical signs, adaptation and clinical application. *Journal of Physiology.* 537 (2), pp. 333-345.

Reilly, T. and Ekblom, B. (2005) The use of recovery methods post-exercise. *Journal of Sports Sciences*. 23 (6), pp. 619-627.

Roberts, L. A. Nosaka, K., Coombes, J. S. and Peake, J. M. (2014) Cold water immersion enhances recovery of submaximal muscle function after resistance exercise. *American Journal of Physiology: Regulatory, Intergrative and Comparative Physiology.* 307, pp. R998-R1008.

Robey, E., Dawson, B., Halson, S., Gregson, W., King, S., Goodman, C. and Eastwood, P. (2013) Effect of evening post exercise cold water immersion on subsequent sleep. *Medicine and Science in Sport and Exercise*. 45 (7), pp. 1394- 1402.

Rossatto, M., de Souza- Bezerra, E. de Ceselles Seixas da Silva, D. A., Avila Santana, T., Rafael Malezam, W. and Carpes, F. P. (2015) Effects of cryotherapy on muscle damage markers and perception of delayed onset muscle soreness after downhill running: A pilot study. *Revista Andaluza de Medicina del Deporte*. 8 (2), pp. 49-53.

Ryschon, T. W., Fowler, M. D., Wysong, R. E., Anthony, A. and Balaban, R. S. (1997) Efficiency of human skeletal muscle in vivo: comparison of isometric, concentric and eccentric muscle action. *Journal of Applied Physiology*. 83 (3), pp. 867-874.

Salman, M. A., Al-Nuwaibit, G., Safar, M. and Al-Mesri, A. (2015) Solubility limits of major salts in sodium chloride solutions. *Journal of Emerging Technology and Advanced Engineering.* 5 (2), pp. 1-7.

Sanchez- Urena, B., Barrantes-Brais, K., Urnea-Bonilla, O., Calleja-Gonzalez, J. and Ostojic, S. (2015) Effect of water immersion on recovery from fatigue: A meta analysis. *European Journal of Human Movement.* 34, pp. 1-14.

Sayers, M. G., Calder, A. M. and Sanders, J. G. (2011) Effect of whole-body contrast-water therapy on recovery from intense exercise of short duration. *European Journal of Sport Sciences*. 11 (4), pp. 293-302.

Schoenfeld, B. J. (2012) The use of nonsteroidal anti-inflammatory drugs for exercise-induced muscle damage: Implications for skeletal muscle development. *Sports Medicine*. 42 (12), pp. 1017-1028.

Scott, W., Stevens, J. and Binder-Macleod, S. A. (2001) Human skeletal muscle fibre type classifications. *Journal of American Physical Therapy Association*. 81, pp. 1810-1816.

Selkow, N. M., Herman, D. C., Liu, Z., Hertel, J., Hart, J. M. and Saliba, S. A. (2015) Blood flow after exercise-induced muscle damage. *Journal of Athletic Training.* 50 (4), pp. 400-406.

Sellwood, K. L., Brukner, P., Williams, D., Nicol, A. and Hinman, R. (2007) Ice-water immersion on delayed-onset muscle soreness: a randomised controlled trial. *British Journal of Sports Medicine*. 41, pp. 392-397.

Selmer, R., Foss, O. P. and Lund-Larsen, P. G. (1990) Reliability of the Reflotron in the determination of cholesterol. *Scandinavian Journal of Clinical and Laboratory Investigation.* 50 (3), pp. 261-271.

Seymour, J. M., Ward, K., Sidhu, P. S., Puthucheary, Z., Steier, J., Jolley, C. J., Rafferty, G., Polkey, M. I. and Moxham, J. (2009) Ultrasound measurement of rectus femoris cross-sectional area and the relationship with quadriceps strength in COPD. *Thorax.* 64, pp. 418-423.

Shin, E. H., Caterson, E. J., Jackson, W. M. and Nesti, L. J. (2014) Quality of healing: defining, quantifying, and enhancing skeletal muscle healing. *Wound Repair and Regeneration.* 22, pp. 18-24.

Sikorski, E. M., Wilson, J. M., Lowery, R. P., Joy, J. M., Laurant, C. M., Wilson, S. M-C., Hesson, D., Naimo, M. A., Averburch, B. and Gilchrist, P. (2013) Changes in perceived recovery status scale following high volume, muscle damaging resistance exercise. *Journal of Strength and Conditioning Research*. 27 (8), pp. 2079-2085.

Sousa, M., Teixeira, V. H. and Soares, J. (2014) Dietary strategies to recover from exercise-induced muscle damage. *International Journal of Food Sciences and Nutrition.* 65 (2), pp. 151-163.

Springer, B. L. and Clarkson, P. M. (2003) Two cases of exertional rhabdomyolysis precipitated by personal trainers. *Journal of the American College of Sports Medicine.* pp. 1499- 1502.

Sramek, P., Simeckova, M., Jansky, L., Savlikova, J. and Vybiral, S. (2000) Human physiological responses to immersion into water of different temperatures. *European Journal of Applied Physiology*. 81, pp. 436-442.

Stacey, D., Gibala, M., Ginis, K. (2010) Effects of recovery method after exercise on performance, immune changes, and physiological outcomes. *Journal of Orthopaedic and Sports Physical Therpay*. 40 (10), pp. 656-665.

Stanley, J., Buchheit, M. and Peake, J. M. (2012) The effect of post-exercise hydrotherapy on subsequent exercise performance and heart rate variability. *European Journal of Applied Physiology.* 112, pp. 951-961.

Stupka, N., Tarnopolsky, M. A., Yardley, N. J. and Phillips, S. M. (2001) Cellular adaptation to repeated eccentric exercise-induced muscle damage. *Journal of Applied Physiology.* 91, pp. 1669-1678.

Takahashi, J., Ishihara, K. and Aoki, J. (2006) Effect of aqua exercise on recovery of lower limb muscles after downhill running. *Journal of Sport Sciences*. 24 (8), pp. 835-842.

Tee, J., Bosch, A. N. and Lambert, M. I. (2007) Metabolic consequences of exercise-induced muscle damage. *Sports Medicine*. 37 (10), pp. 827-836.

Telley, I. A., Stehle, R., Ranatunga, K. W., Pfitzer, G., Stussi, E. and Denoth, J. (2006) Dynamic behaviour of half-sarcomeres during and after stretch in activated rabbit psoas myofibrils: sarcomere asymmetry but no 'sarcomere popping'. *Journal of Physiology.* 573 (1), pp. 173-185.

Tidball, J. G. (2005) Inflammatory processes in muscle injury and repair. *American Journal of Physiology: Regulatory, Integrative and Comparative Physiology.* 288, pp. R345- R353.

Tiidus, P. M. (1997) Manual massage and recovery of muscle function following exercise: A literature review. *Journal of Orthopaedic and Sports Physical Therapy.* 25 (2), pp. 107-112.

Tomlin, D. L. and Wenger, H. A. (2001) The relationship between aerobic fitness and recovery from high intensity intermittent exercise. *Sports Medicine.* 31 (1), pp. 1-11.

Torres, R., Ribeiro, F., Duarte, J. A. and Cabri, J. M. H. (2012) Evidence of physiotherapeutic interventions used currently after exercise-induced muscle damage: Systematic review and meta-analysis. *Physical Therapy in Sport.* 13, pp. 101-114.

Torres-Ronda, L. and Alcazar, X. S. (2014) The properties of water and their applications for training. *Journal of Human Kinetics*. 44 (3), pp. 237-248.

Toumi, H., and Best, T. M. (2003) The inflammatory response: friend or enemy for muscle injury? *British Journal of Sports Medicine.* 37, pp. 284- 286.

Tseng, C. Y., Lee, J. P., Tsai, T. S., Lee, S. D., Kao, C. L., Liu, T. C., Lai, C. H., Brennan Harris, M. and Kuo, C. H. (2013) Topical cooling (icing) delays recovery from eccentric exercise-induced muscle damage. *Journal of Strength and Conditioning Research*. 27 (5), pp. 1354-1361.

Umbel, J. D., Hoffman, R. L., Dearth, D. J., Chlebourn, G. S., Manini, T. M. and Clark, B. C. (2009) Delayed-onset muscle soreness induced by low-load blood flow-restricted exercise. *European Journal of Applied Physiology*. 107, pp. 687-695.

Vaile, J. M., Gill, N. D. and Blazevich, A. J. (2007) The effect if contrast water therapy on symptoms of delayed onset muscle soreness. *Journal of Strength and Conditioning Research.* 21 (3), pp. 697-702.

Vaile, J., Halson, S. and Gill, N. (2008) Effect of cold water immersion on repeat cycling performance and thermoregulation in the heat. *Journal of Sports Science.* 26 (5), pp. 431-440.

Vaile, J., Halson, S., Gill, N. and Dawson, B. (2008) Effect of hydrotherapy on the signs and symptoms of delayed onset muscle soreness. *European Journal of Applied Physiology.* 102, pp. 447-455.

Vaile, J., Halson, S., Gill, N. and Dawson, B. (2008) Effect of hydrotherapy on recovery from fatigue. *International Journal of Sports Medicine.* 29, pp. 539-544.

Vaile, J., O'Hagan, C., Stefanovic, B., Walker, M., Gill, N. and Askew, C. D. (2011) Effect of cold water immersion on repeated cycling performance and limb blood flow. *British Journal of Sports Medicine*. 45, pp. 825-829.

Versey, N., Halson, S. and Dawson, B. (2011) Effect of contrast water therapy duration on recovery of cycling performance: a dose-response study. *European Journal of Applied Physiology.* 111, pp. 37-46.

Versey, N., Halson, S. and Dawson, B. T. (2012) Effect of contrast water therapy duration on recovery of running performance. *International Journal of Sports Physiology and Performance.* 7, pp. 130-140.

Versey, N. G., Halson, S. L. and Dawson, B. T. (2013) Water immersion recovery for athletes: Effect on exercise performance and practical recommendations. *Sports Medicine.*

Warden, S. J. (2010) Prophylactic use of NSAIDs by athletes: A risk/benefit assessment. *The Physician and Sports Medicine*. 38 (1), pp. 1-7.

Warren, G. L., Ingalls, C. P., Lowe, D. A. & Armstrong, R. B. (2001). Excitation-contraction uncoupling: major role in contraction-induced muscle injury. *Exercise and Sport Sciences Reviews*. 29, pp. 82–87.

Weerapong, P., Hume, P. A. and Kolt, G. S. (2005) The mechanisms of massage and effects on performance, muscle recovery and injury prevention. *Sports Medicine.* 35 (3), pp. 235-256.

White, G. E. and Wells, G. D. (2013) Cold-water immersion and other forms of cryotherapy: physiological changes potentially affecting recovery from high intensity exercise. *Extreme Physiology and Medicine*. 2 (26), pp. 1-11.

White, G. E., Rhind, S. G. and Wells, G. D. (2014) The effect of various cold water immersion protocols on exercise-induced inflammatory response and functional recovery

from high-intensity sprint exercise. *European Journal of Applied Physiology*. 114, pp. 2353-2367.

Wilcock, I. (2005) *The effect of water immersion, active recovery and passive recovery on repeated bouts of explosive exercise and blood plasma fraction.* Masters of Health Science Thesis. Auckland University of Technology.

Wilcock, I. M., Cronin, J. B. and Hing, W. A. (2006) Physiological response to water immersion: A method for sport recovery. *Sports Medicine*. 36 (9), pp. 747-765.

Wilcock, I. M., Croning, J. B. and Hing, W. A. (2006) Water immersion: Does it enhance recovery from exercise? *International Journal of Sports Physiology and Performance.* 1, pp. 195-206.

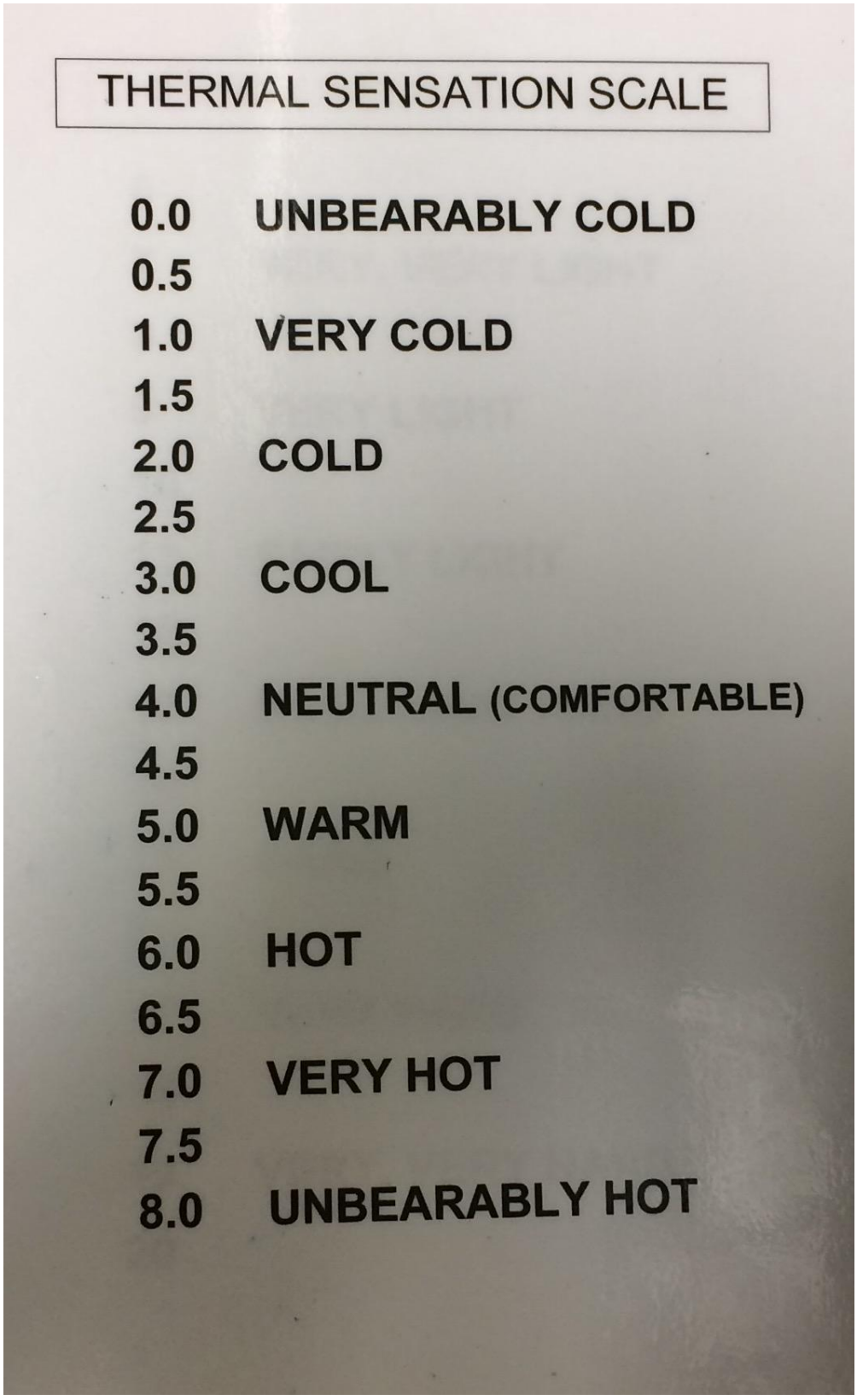
Willems, M. E. T. and Northcott, S. R. (2009) Gender differences after downhill running for voluntary isometric contractions of the knee extensor muscles using surface EMG. *Medicina Sportiva*. 13 (1), pp. 35-42.

Xing, Z., Gauldie, G., Cox, H., Baumann, M., Jordana, M., Lei, X. F. and Achong, M.K. (1998) IL-6 is an anti-inflammatory cytokine required for controlling local or systemic acute inflammatory responses. *Journal of Clinical Investment.* 101, pp. 311-320.

Zusman, M. (1986) The absolute visual analogue scale (AVAS) as a measure of pain intensity. *The Australian Journal of Physiotherapy*. 32 (4), pp. 244-246.

APPENDICES

Appendix A: Thermal Sensation Scale



Appendix B: Visual Analogue Scale- Pain

Visual Analogue Scale

NO PAIN

WORST POSSIBLE PAIN

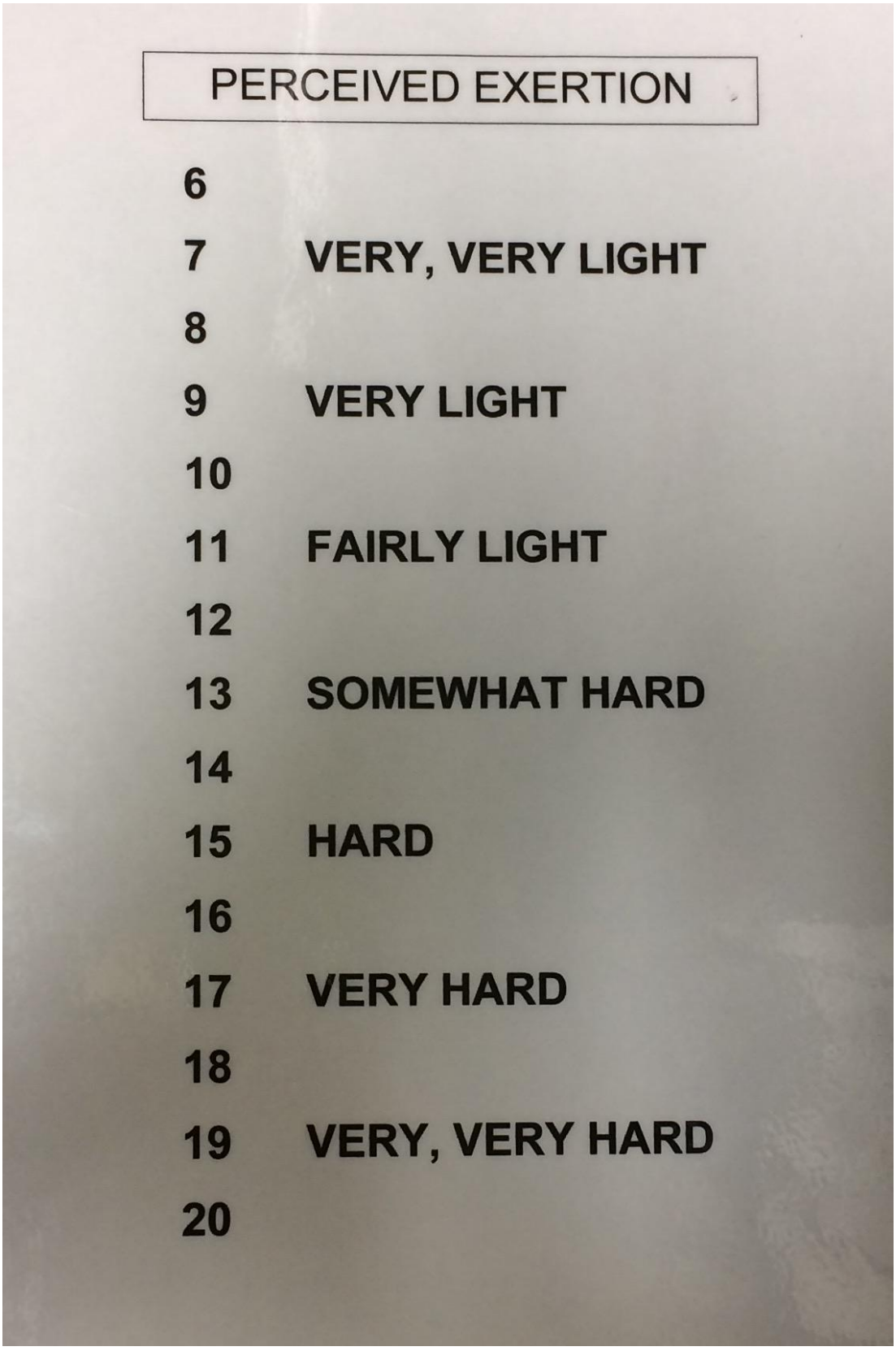
Appendix C: Visual Analogue Scale- Recovery

Visual Analogue Scale

NOT AT ALL RECOVERED

FULLY RECOVERED

Appendix D: Rate of perceived exertion (RPE) scale

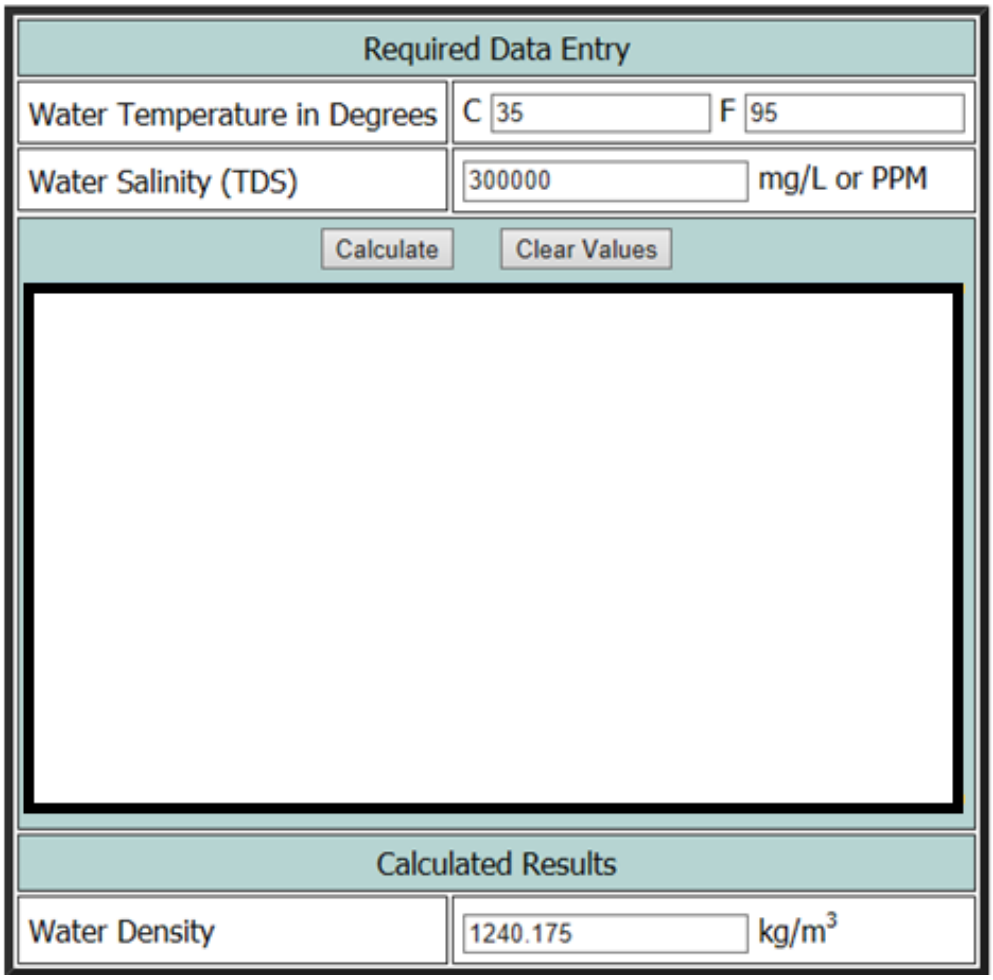


Appendix E: Andy Murray pictured in an ice bath following Wimbledon victory in June 2016

Appendix F: Calculating Density

Calculating the density of a saline solution

http://www.csgnetwork.com/h2odenscalc.html



Calculating the density of water from 0°C to 100°C

http://www.vaxasoftware.com/doc_eduen/qui/denh2o.pdf

Density of liquid water from 0 °C to 100 °C

www.vaxasoftware.com

External pressure: 1 atm = 101 325 Pa

Temperature °C	Density kg/m^3	Temperature °C	Density kg/m^3	Temperature °C	Density kg/m^3
0 (ice)	917.00	33	994.76	67	979.34
0	999.82	34	994.43	68	978.78
1	999.89	35	994.08	69	978.21
2	999.94	36	993.73	70	977.63
3	999.98	37	993.37	71	977.05
4	1000.00	38	993.00	72	976.47
5	1000.00	39	992.63	73	975.88
6	999.99	40	992.25	74	975.28
7	999.96	41	991.86	75	974.68
8	999.91	42	991.46	76	974.08
9	999.85	43	991.05	77	973.46
10	999.77	44	990.64	78	972.85
11	999.68	45	990.22	79	972.23
12	999.58	46	989.80	80	971.60
13	999.46	47	989.36	81	970.97
14	999.33	48	988.92	82	970.33
15	999.19	49	988.47	83	969.69
16	999.03	50	988.02	84	969.04
17	998.86	51	987.56	85	968.39
18	998.68	52	987.09	86	967.73
19	998.49	53	986.62	87	967.07
20	998.29	54	986.14	88	966.41
21	998.08	55	985.65	89	965.74
22	997.86	56	985.16	90	965.06
23	997.62	57	984.66	91	964.38
24	997.38	58	984.16	92	963.70
25	997.13	59	983.64	93	963.01
26	996.86	60	983.13	94	962.31
27	996.59	61	982.60	95	961.62
28	996.31	62	982.07	96	960.91
29	996.02	63	981.54	97	960.20
30	995.71	64	981.00	98	959.49
31	995.41	65	980.45	99	958.78
32	995.09	66	979.90	100	958.05

www.vaxasoftware.com